ISSN: 0975-833X

Available online at http://www.journalcra.com

International Journal of Current Research
Vol. 10, Issue, 12, pp.76638-76642, December, 2018

DOI: https://doi.org/10.24941/ijcr.33673.12.2018

INTERNATIONAL JOURNAL OF CURRENT RESEARCH

RESEARCH ARTICLE

COMPARATIVE EVALUATION OF CONCENTRATED GROWTH FACTOR (CGF) AND PLATELET RICH FIBRIN (PRF) IN COMBINATION WITH DFDBA AND OPEN FLAP DEBRIDEMENT WITH PRF FOR THE FURCATION DEFECTS (GRADE II AND GRADE III)

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ARTICLE INFO

Article History:
Received 14th September, 2018
Received in revised form
21st October, 2018
Accepted 07th November, 2018
Published online 31st December, 2018

Key Words:
Decalcified freeze dried bone graft, Concentrated growth factor (CGF), Platelet Rich fibrin (PRF).

ABSTRACT

Background: Furcation area offer unique and challenging problems, creates situations in which routine periodontal procedures are somewhat limited and special procedures are generally required.
Materials and Methods: This study is comparative evaluation of DFDBA+ CGF +PRF versus open flap debridement with PRF in grade II/III furcation with follow up of 12 months. The study population included 30 subjects, who visited the out-patient department of periodontics, Govt Dental College and Hospital Srinagar with complaint of bleeding and sensitivity, diagnosed as chronic periodontitis. After conducting routine examinations, Relative attachment and probing depth were measured. The measurement of Relative attachment was made with ocllusal stent as guiding value. Probing depth was made with UNC-15 periodontal probe. Furcation depth was measured with Probe (UNC-15). Furcation defect was filled with CGF+PRF+DFDBA in test group and open flap debridement with PRF in control group, flap was coronally advanced in both test and in control group.
Results: Concentrated growth factor and PRF with DFDBA have been shown to be a promising and successful approach for the treatment of furcation defect. Its gaining clinical attachment significantly manages both the gingival recession and furcation involvement simultaneously.
Conclusion: Within the limitation of this study, significant improvement with autologous CGF+PRF+DFDBA implies its role as a regenerative material in the treatment of furcation defects.

Citation: Dr. Gazanfer Ali Shah, Dr. Suhail Majid Jan and Dr. Reyaz Ahmad Mir. **2018.** "Comparative evaluation of concentrated growth factor (cgf) and platelet rich fibrin (prf) in combination with dfdba and open flap debridement with prf for the furcation defects (grade II and grade III)", *International Journal of Current Research*, 10, (12), 76638-76642.

INTRODUCTION

Periodontal tissue is destroyed in the course of Periodontitis by disproportionate immunologic responses to a triggering agent, such as bacteria in biofilm (Taubman, 2005). It has been reported that molars with furcation involved caused by periodontitis, have a higher rate of periodontal breakdown and respond less favorably to periodontal therapy than molars without furcation involvement or single-rooted teeth (Kalkwarf 1988; Lavanchy, 1987 and Goldman *et al*., 1986). Multirooted teeth offer unique and challenging problems for the periodontist. The furcation area, because of the interrelationships between the size and shape of the teeth, the roots and their alveolar housing, and the varied nature and pattern of periodontal destruction create situations in which routine periodontal procedures are somewhat limited and special procedures are generally required.

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It has been shown that the best chance for success lies in early recognition and treatment of furcation involvement (Wærhaug, 1980). Grade II furcation is any involvement of the interradicular bone without a through-and-through ability to probe, Grade III is through and through without soft tissue recession. Various materials have been used to resolve furcation defect including autografts (Schallhorn, 1970; Schallhorn, 1977; Bowers, 1989; Bowers, 1989 and Bowers, 1989), demineralised freeze-dried bone allografts (DFDBAs) (Tsao, 2016 and Hoffmann, 2006), bovine-derived xenografts, barrier membranes and combinations of membranes and bone grafts. Although these regenerative materials are being used today, the introduction of biomimetic agents such as enamel matrix derivatives (Hoffmann, 2006), Concentrated growth factor (CGF) (Nityasri. 2018) platelet rich plasma (PRP) (Choukroun, 2001), platelet-derived growth factor and bone morphogenetic proteins have shown better outcomes in furcation treatment. Concentrated growth factor (CGF) was first developed by Sacco (2006), (Jaishree, 2017) is a relatively

new technology within the area of regenerative medicine. (CGF) is an advanced second generation platelet concentrate, obtained with differential continuous centrifugal technology, containing many kinds of growth factors and fibrins, and able to facilitate the recovery of soft and hard tissues. CGF is different from platelet-rich plasma (PRP) and platelet-rich fibrin (PRF) in the methods for production because no additives are added during its production. CGF has a higher adhesive strength, tensile strength, higher viscosity than the other platelet preparations. CGF has a difference in centrifugation speed which permits the isolation of much larger and denser fibrin matrix richer in growth factors. CGF is a fibrin rich organic matrix which contains growth factors, platelets, leukocytes and CD34+ stem cells which help in the process of regeneration and also has immunological cells that are effective in regulating inflammation and minimizing the risk of infection. (CGF) is a biological inducing material which improves the quality of the formed bone, and facilitates the formation of bones and the healing of tissues. Platelet-rich fibrin (PRF) developed in France by Choukroun *et al.* (Choukroun, 2001), is a second generation platelet concentrate. Its advantages over the better known platelet-rich plasma (PRP) include ease of preparation/application, minimal expense and lack of biochemical modification (no bovine thrombin or anticoagulant is required). PRF is a strictly autologous fibrin matrix containing a large quantity of platelet and leukocyte cytokines (Hoffmann, 2006; Choukroun, 2001; Pradeep, 2009; Dohan Ehrenfest, 2009; Dohan *et al*., 2006 and Dohan, 2006).

Filling of the furcation periodontal defects with various types of bone grafts is one of the most widely employed techniques aimed at restoring the lost periodontal attachment apparatus (Brunsvold and Mellonig 1993). The use of intra- or extraoral autografts and demineralized freeze-dried bone allograft (DFDBA) may lead not only to clinical improvements in term of probing depth (PD) reductions and gains of clinical attachment, but also histologically by the formation of a new connective tissue attachment (i.e. new cementum with inserting collagen fibers) and new alveolar bone (Dragoo and Sullivan 1973, Bowers *et al*. 1989, Brunsvold and Mellonig 1993). CGF and PRF combination offers unique advantage in inducing bone growth and periodontal regeneration with DFDBA in comparison to when using DFDBA alone. The coronally advanced flap is a simple pedicle flap that can be utilized for root coverage, and does not require graft harvesting. A potential limitation of the coronally advanced flap is the limited gain in the apico-coronal dimension of the keratinized tissue which is an important parameter in preventing the recurrence.

MATERIALS AND METHODS

This study is comparative evaluation of DFDBA+PRF+CGF versus open flap debridemen with PRF in grade II/III furcation. The study population included 30 subjects, who visited the out-patient department of periodontics, Govt Dental College and Hospital Srinagar with complaint of bleeding and sensitivity, diagnosed as chronic periodontitis. 15 subjects received treatment with DFDBA+CGF+PRF in combination and 15 subjects received Open flap debridement with PRF only, both were followed by coronal advancement flap. After conducting routine examinations, Relative attachment and probing depth were measured. The measurement of Relative attachment was made with occlusal stent as guiding value. Probing depth was made with UNC-15 periodontal probe. Furcation depth was measured with Probe (UNC-15). Furcation defect was filled with CGF+PRF+DFDBA and flap was coronally advanced in test group and in control group open flap debridement with PRF was done. Inclusion criteria for the study were as follows (1) no systemic diseases that could influence the outcome of the therapy, (2) a good level of oral hygiene, (3) compliance with the maintenance program and Grade II/III buccal furcation defects with associated probing depths >6.0 mm following initial non-surgical therapy. All patients completed a comprehensive medical and dental history examination. Patients were then given an explanation of the study purpose including benefits and associated risks, as well as alternative treatment regimens. Finally, a signed informed consent was obtained. All patients received initial therapy including full mouth scaling and root planing, oral hygiene instructions and occlusal adjustment addressed when indicated. Approximately 4–6 weeks following the oral hygiene phase therapy, all patients underwent re-evaluation examination to assess PD, attachment level, furcation involvement, and bleeding on probing, following which they were approved for flap surgery. The following clinical parameters were assessed at baseline and 12 months after the surgical procedure (1)) Probing pocket depth (PD), 2. Relative attachment level 3. The cast model of the patient was made on which cold cure acrylic stent was prepared. Stent was prepared for assessing relative attachment level. The bone defect was measured from furcation entrance to base of defect at baseline, and 12 months using UNC-15 probe.

Pre-Surgical Therapy

That patient underwent Scaling and Root Planning by ultrasonic dental unit and Gracey curettes (Hu Friedy) under local anaesthesia instructions regarding oral hygiene maintainance, flossing were given to patient prior to surgery. CGF is an autologous preparation; about 9 ml of patient's venous blood is collected without anticoagulant solutions in sterile Vacuette tubes. The tubes are kept for centrifugation (220 v ± 10 % Hz) with one step centrifugation protocol: 30sec -acceleration, 2min - 2700 rpm, 4min 2400 rpm, 4min - 2700 rpm, 3min - 3000 rpm, 36sec – deceleration and stop. This results in 4 different phases.

Phases of CGF

1. **Superior phase** – Serum
2. **Interim phase** – Fibrin buffy coat
3. **Liquid phase** – Growth factors
4. **Lower phase** – Red blood cells.

For the preparation of platelet rich fibrin centrifugation machine, 5ml syringe and 9ml blood collection tubes were used. Five milliliter blood was collected from the same patient. Then, the blood was drawn into the tube without anticoagulant. The blood was transferred to centrifugation machine within 1-2 minutes after collection and it centrifuged for 15 minutes at 3000rpm. Absence of anticoagulant allows activating the majority of the platelets contained in the sample which triggers a coagulation cascade. Fibrinogen initially concentrates in the upper part of the tube; until the effect of the circulating thrombin transforms it into a fibrin network. The PRF was collected from centrifuge tube with tweezer and placed on a sterile gauze piece.

The surgical site was isolated and anaesthetized with 2% xylocaine hydrochloride with adrenaline (1:200000. Crevicular incision was made from first premolar to 2nd molar. The full thickness flap was then raised beyond the mucogingival junction. The furcation area was debrided thoroughly, and granulation tissue was removed through scaling and root planning. After scaling and root planning DFDBA granules were mixed with PRF and CGF and placed in the furcation defect area. After the DFDBA granules with PRF+CGF were placed, PRF membrane was placed to cover the site and the osseous graft. The patient was evaluated at baseline and and 12 months with a positive result when grade-II furcation defect was treated with PRF+CGF+DFDBA than with PRF only.

Postoperative care

All patients received analgesics and antibiotics for 1 week (500 mg amoxicillin/day). The postoperative care consisted of 0.2% Chlorhexidine rinses twice a day for 4 weeks. Post-operative appointments were carried out at 7–10 days, 1 month and 12 months. At 1 and 12 month recall, Relative attachment level, probing pocket depth, reduction of furcation depth were recorded. Assessment of soft tissue findings revealed, after treatment, pocket depth was significantly decreased as compared to baseline values in both groups, but the difference was not significant in control group. The mean of periodontal probing depth (PD) for DFDBA+ PRF+CGF was 9.8 mm ± 1.83 at baseline and 4.4 mm ± 1.07 at 12 months, while in the open flap debridement with PRF group, mean of probing pocket depth (PD) was 9.7 mm ± 1.56 at baseline and 7.3 mm ± 1.89 at 12 months, showing statistically significant result in test group. Relative attachment level depicted 13.5 ±2.22 at baseline and 7.5 ± 1.90 after 12 months, showed gain of 6.0 ± 0.32 and in control group it showed 13.5 ± 2.22 at baseline and 10.0 ±1.63 after 12 months, depicted gain of 3.5 ± 0.59. Reduction in furcation depth in test group as shown in table 3 is 4.22±0.01 and in control group it is 2.27±0.11.

Statistical Observation

Table 1. Probing pocket depth (DFDBA +PRF +CGF versus Open flap debridement with PRF)

Time Interval	Test Group	Control Group	p-value
T1	9.8 +- 1.83	9.7 +- 1.56	
T2	4.4 +- 1.07	7.3 +- 1.89	
T1-T2	5.4 +- 0.76	2.4 +- 0.77	1.22234E-05= 0.00012

P-value <0.001 (Statistically significant)

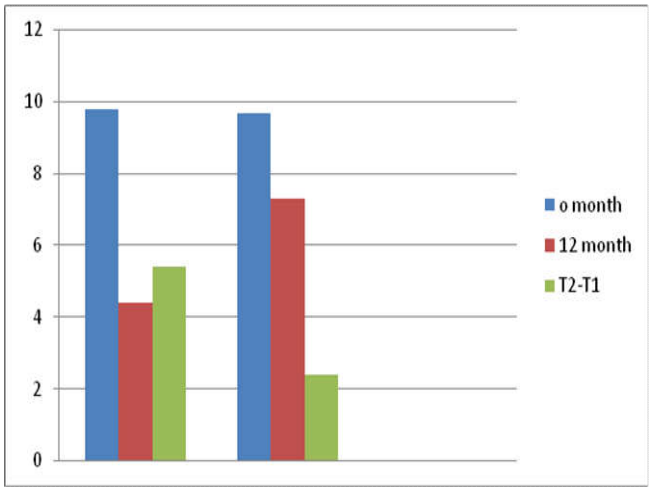


Table 2. Relative attachment level (DFDBA+PRF+CGF versus open flap debridement with PRF)

Time Interval	Test Group	Control Group	p-value
T1	13.5 +- 2.22	13.5 +- 2.22	
T2	7.5 +- 1.90	10.0 +- 1.63	
T1-T2	6.0 +- 0.32	3.5 +- 0.59	0.000772

P-value < 0.001 (Statistically significant)

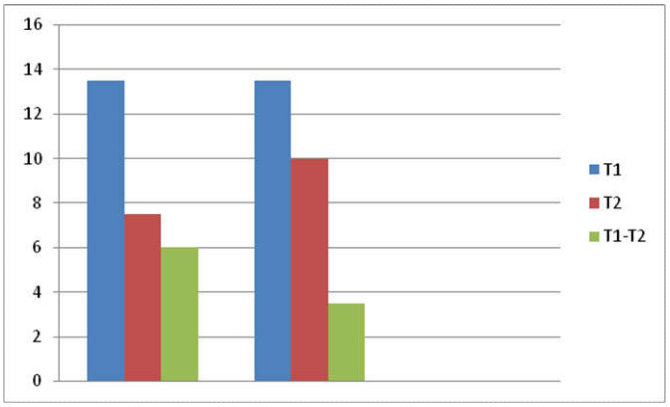
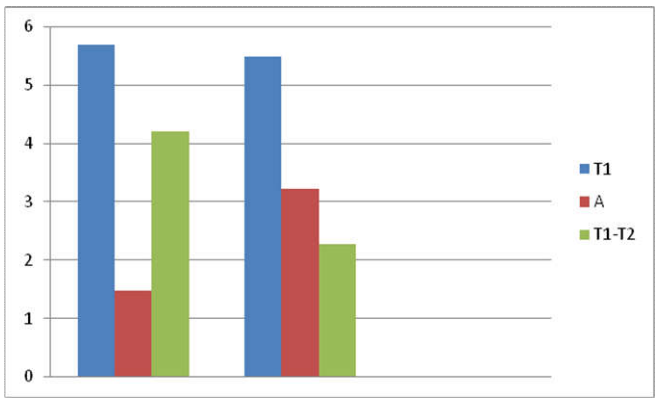


Table 3. Reduction in furctaion depth penetration on probing

Time Interval	Test Group	Control Group	P value
T1	5.70±0.78	5.50±0.89	
T2	1.48±0.77	3.23±0.78	
T1-T2	4.22±0.01	2.27±0.11	0.000878

P-value < 0.001 (Statistically significant)



DISCUSSION

Periodontal disease is among the most prevalent diseases worldwide and is characterized by the presence of gingival inflammation, periodontal pocket formation, loss of periodontal attachment and loss of alveolar bone around the affected teeth (Rosenberg, 1998 and Kanakamedala *et al*., 2009). The goal of periodontal therapy includes not only the arrest of periodontal disease progression, but also the regeneration of structures lost due to disease (Urist, 1965). Bone grafting is one of the most common forms of regenerative therapy and is usually essential for restoring periodontal supporting tissue. A wide range of bone grafting materials, including bone grafts and bone graft substitutes, have been applied and evaluated clinically, including autografts, allografts, xenografts, and alloplasts (synthetic/semisynthetic materials). DFDBA is widely used in periodontal therapy and has been demonstrated to be safe and capable of inducing new bone formation. DFDBA is shown to be both osteoconductive and osteoinductive. Urist *et al*. showed through numerous animal experiments that DFDBA could stimulate the formation of new bone by osteoinduction.

That is, the graft material induces host undifferentiated mesenchymal cells to differentiate into osteoblasts with subsequent formation of new bone. Moreover, DFDBA also provides a scaffold for osteoconduction. DFDBAs have repeatedly demonstrated significant improvements in soft and hard tissue clinical parameters for the treatment of intraosseous periodontal defects. Recently, the use of growth factors in periodontal regeneration has shown promising results. Growth factors are a class of natural biologic mediators that regulate key cellular events in tissue regeneration including cell proliferation, chemotaxis, differentiation, and matrix synthesis via binding to specific cell surface receptors.[25] Platel*et al*pha (α) granules form an intracellular storage pool of growth factors including platelet-derived growth factor, transforming growth factor β (including β-1 and β-2 -isomers), vascular endothelial growth factor, and epidermal growth factor and insulin-like growth factor-1. A combination of PRF+CGF with DFDBA demonstrated better results in probing pocket depth reduction, reduction in depth of furcation depth and clinical attachment level gain as compared to open flap debridement with PRF in the treatment of periodontal furcation defects. In this study, Assessment of soft tissue findings revealed, after treatment, pocket depth was significantly decreased as compared to baseline values in both groups, but the difference was not significant in control group. The mean of periodontal probing depth (PD) for DFDBA+ PRF+CGF was 9.8 mm ± 1.83 at baseline and 4.4 mm ± 1.07 at 12 months, while in the open flap debridement group with PRF, mean of probing pocket depth (PD) was 9.7 mm ± 1.56 at baseline and 7.3 mm ± 1.89 at 12 months, showing statistically significant result in test group. Relative attachment level depicted 13.5 ± 2.22 at baseline and 7.5 ± 1.90 after 12 months, showed gain of 6.0 ±0.32 and in control group it showed 13.5 ± 2.22 at baseline and 10.0 ± 1.63 after 12 months, depicted gain of 3.5 ± 0.59. Reduction in furcation depth in test group as shown in table 3 is 4.22±0.01 and in control group it is 2.27±0.11.

This result may be attributed to beneficial effects of PRF+CGF on DFDBA. Concentrated growth factor (CGF) was first developed by Sacco (2006), is a relatively new technology within the area of regenerative medicine. (CGF) is an advanced second generation platelet concentrate, obtained with differential continuous centrifugal technology, containing many kinds of growth factors and fibrins, and able to facilitate the recovery of soft and hard tissues. CGF is different from platelet-rich plasma (PRP) and platelet-rich fibrin (PRF) in the methods for production because no additives are added during its production. CGF has a higher adhesive strength, tensile strength, higher viscosity than the other platelet preparations. CGF has a difference in centrifugation speed which permits the isolation of much larger and denser fibrin matrix richer in growth factors. CGF is a fibrin rich organic matrix which contains growth factors, platelets, leukocytes and CD34+ stem cells which help in the process of regeneration and also has immunological cells that are effective in regulating inflammation and minimizing the risk of infection. (CGF) is a biological inducing material which improves the quality of the formed bone, and facilitates the formation of bones and the healing of tissues. PRF has been introduced by Choukroun *et al*. in 2001. PRF consists of a fibrin matrix polymerised in a tetramolecular structure; the incorporation of platelets, leukocytes, and cytokines; and circulating stem cells. Slow fibrin polymerization during PRF processing leads to the intrinsic incorporation of platelet cytokines and glycan chains in the fibrin meshes.

This result implies that PRF, unlike the other platelet concentrates, is able to progressively release cytokines during fibrin matrix remodeling. It is also found that PRF organizes as a dense fibrin scaffold with a high number of leukocytes concentrated in one part of the clot. Leukocytes seem to have a strong influence on growth factor release, immune regulation, anti-infectious activity, and matrix remodeling during healing. It is an optimal matrix for migration of endothelial cells and fibroblasts. It permits a rapid angiogenesis and an easier remodeling of fibrin in a more resistant connective tissue. Such a mechanism might explain the clinically observed soft tissue healing properties of PRF. However, histological studies are needed to establish the exact nature of this clinical attachment gain. In our study, PRF does not seem to have any additional favorable effect on defect fill and defect resolution in the treatment of periodontal furcation defects. However, various in vitro studies have shown a beneficial effect of PRF on bone healing like its effect on proliferation and differentiation on osteoblasts (Ehrenfest *et al*. 2009, He *et al*. 2009). When mixed with the graft, PRF fragments serve as a biological connector between bone particles. Moreover, the gradual release of cytokines plays a significant role in the selfregulation of inflammatory and infectious phenomena within the grafted material. Thus, in the present study PRF+CGF with DFDBA demonstrated better results in probing pocket depth reduction and clinical attachment level gain as compared to open flap debridement with PRF alone as depicted in Table 1 and Table 2 and Table 3 in the treatment of periodontal furcation defects. This result may be attributed to beneficial effects of PRF+CGF in combination with DFDBA. However, histological studies are needed to establish the exact nature of this clinical attachment gain. However, various in vitro studies have shown a beneficial effect of PRF and CGF on bone healing. Within limits of the study, it can be concluded that future longterm studies with a large sample size and utilization of advanced radiological techniques should be carried out to further explore the role of PRF+CGF induction with DFDBA in the management of periodontal furcation defects.

Conclusion

Periodontal surgical procedures using growth factors are a new method that accelerates and enhances the process of natural wound healing and bone regeneration mechanism. Concentrated Growth Factor (CGF) is a biological repairing material, a new generation of blood extract, which stimulates and accelerates the bone formation and healing of tissues. CGF also improves the quality of the formed bone, enhances tissue regeneration, promotes stabilization of grafts and is effective in regulating inflammation. In combination with PRF it has additional role of induction to DFDBA for bone formation. Positive results of CGF+PRF+DFDBA versus open flap debridement with PRF in this study as depicted in statistical results favours its use for furcation involved teeth. The success of this therapy lies in the local delivery of a high concentration of growth factors and proteins. This is similar to the physiologic wound healing, and supports reparative tissue process and local infiltration therapy, taking the surgical practice of regenerative techniques to a higher level.

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