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Research Article

Comparative Evaluation of Efficacy of Platelet Rich Fibrin, DFDBA and in Combination of Both in Healing of Periapical Defects-An *In-vivo* Study

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Abstract

Introduction: The main objective of this study is to evaluate the efficacy of PRF, DFDBA and combination of both in periapical defects healing.

Materials and Methods: 24 patients, aged between 20 - 40 years, were screened for bilateral periapical lesions of maxillary lesions below 2 cm in dimension in the radiograph and were included in the study. The patients were divided into 3 Groups with 8 in each, group 1: One lesion taken as control, another lesion treated with PRF, group 2: One lesion taken as control, another lesion treated with DFDBA, group 3: One lesion taken as control another lesion treated with combination of DFDBA and PRF. Patients recalled after 3 months and 6 months for clinical and radiographic evaluation followed by Morphometric area analysis (MA).

Results: On comparing percentage in bone formation from baseline to 6 months in Test vs Control showed more percentage change with Group 3 test containing both PRF and DFDBA.

Conclusion: Group III containing combination of PRF and DFDBA showed better results than other two groups containing PRF and DFDBA individually, but further long-term follow up as well as histological nature of newly formed tissues by either treatment remains to be elucidated.

Keyword: *Platelet Rich Fibrin; DFDBA; Peri-radicular Surgery; Regenerative Endodontics*

Introduction

Peri-radicular surgery is an established treatment option in endodontics for management of periapical pathologies. The amount and location of the bone adjacent to root structures effect the prognosis of peri-radicular surgeries [1]. Wound healing occurs by repair and regeneration. Regenerative surgery, including the use of barrier membranes and graft materials, can reduce probing depths, support formation of periodontal ligament and allow regenerative rehabilitation and functional reconstruction [2]. Platelet rich fibrin (PRF) induction is a way to accelerate and enhance the body's natural wound healing mechanism [3]. PRF is a rich source of Platelet Derived Growth Factor (PDGF), Transforming Growth Factor (TGF) and Insulin Like Growth Factor (IGF), etc. TGF-stimulates bio-synthesis of type I collagen and induces deposition of bone matrix. IGF and PDGF stimulates bone formation by proliferation and differentiation, and it is synthesized and secreted by osteoblasts [4]. Allogenic bone grafts have been used during the last 3 decades as either freeze-dried bone allograft (FDBA) or

demineralized freeze-dried bone allograft (DFDBA). Both types of allografts have been successfully used for regeneration of the supporting periodontium. These grafting materials have different mechanisms of action. FDBA creates an osteoinductive mesh and allows resorption when implanted in mesenchymal tissues. DFDBA also provides an osteoinductive surface and stimulates mesenchymal cell migration, fixation, and osteogenesis, when implanted in well-vascularized bone and also induces endochondral bone formation when implanted in tissues that otherwise would not form bone [5].

Purpose of the Study

The purpose of this study was to evaluate the efficacy of PRF, DFDBA and combination of PRF and DFDBA in promoting bone regeneration in periapical defects radiographically with 3 months and 6 months follow up postoperatively in 24 patients.

Materials and Methods

The present *in vivo* study was designed to evaluate the efficacy of platelet rich fibrin, DFDBA, and combination of both in periapical defects. 24 patients, aged between 20 - 40 years, were patients were initially screened for bilateral periapical lesions of maxillary central incisors both clinically and radiographically. Only lesions below 2 cm in dimension in the radiograph were included in the study. The patients were divided into 3 Groups with 8 in each:

- **Group 1**: One lesion taken as control, another lesion treated with PRF.
- **Group 2:** One lesion taken as control, another lesion treated with DFDBA.
- **Group 3**: One lesion taken as control another lesion treated with combination of DFDBA and PRF.

The patient was explained about the whole surgical procedure. The treatment was done after getting informed consent from the patient. Radiographic angulations were standardized for subsequent follow up during the period of the study. The root canal treatment was completed prior to surgery. In order to reduce postoperative pain and swelling, all patients were advised to take an NSAID prior to surgical procedure. Following administration of local anesthesia, limited thickness mucoperiosteal flap Luebke-Ochsenbein Flap reflection is done by scalloped horizontal incisions extend from a point 1 - 2 mm short of entering mucobuccal fold to the point to the attached gingival 3 - 5 mm above the marginal gingival and sulcus depth. Endometrics was used as transfer of record from a periapical radiograph on the soft tissues in the surgical area both while placing an incision and while carrying out osteotomy procedure. High Torque, Low Speed Handpiece with external coolant was used to do osteotomy. Apical curettage is done with curette. Apical 3mm of root end resection is done. After root end preparation GIC placed in all cases as root end filling material. Just before surgery, intravenous blood (by venipuncturing of the antecubital vein) was collected in three 10-mL sterile tubes without anticoagulant and immediately centrifuged in a centrifugation machine at 3,000 revolutions per minute for 10 minutes. Blood centrifugation immediately after collection allows the composition of a structured fibrin clot in the middle of the tube, just between the red corpuscles at the bottom and acellular plasma (platelet-poor plasma [PPP]) at the top. PRF was easily separated from red corpuscles base (preserving a small red blood cell [RBC] layer) using sterile tweezers and scissors just after removal of PPP and then transferred onto a sterile compress. A stable fibrin membrane was obtained by squeezing serum out of the PRF clot. Test material PRF or DFDBA or Combination of PRF and DFDBA is placed on one tooth and another tooth is taken as control, in all the three groups according to randomization. A 3-0 silk non-absorbable sutures were used for suturing. After re-approximation interrupted sutures are placed. Post-operative instructions were given, followed by post-operative radiograph. Suture removal was done after 5 days. Patients recalled after 3 months and 6 months for clinical and radiographic evaluation (Figure 1 and 2). Morphometric area analysis (MA) was done three times i.e. baseline,3 months and 6 months postoperatively by using commercially available image processing software (Adobe Photoshop® 6.0, Adobe Systems, San Jose, USA) and periapical area calculation was done with open source software-ImageJ (Research Services Branch, NIH, Bethesda, Maryland, USA) (Figure 3 and 4).

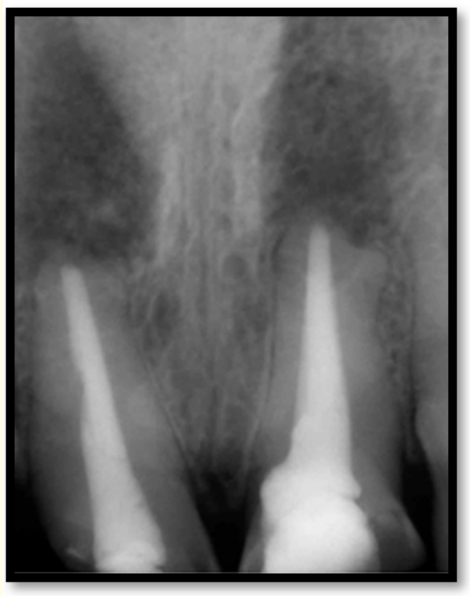

Figure 1: Group 3 pre-operative radiograph showing the bilateral periapical lesion.

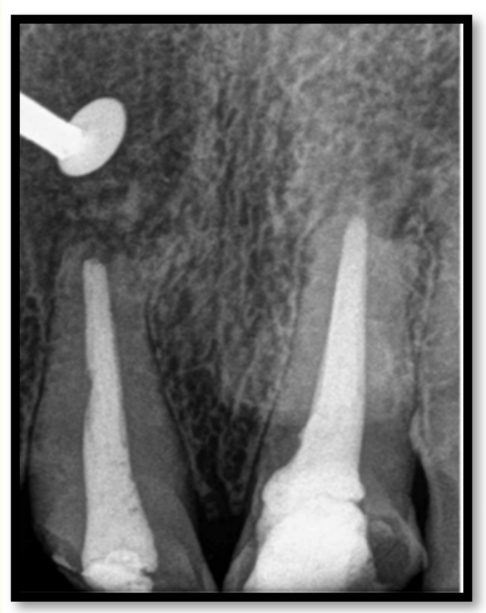

Figure 2: Group 3 post-operative 6 months follow up radiograph showing periapical healing.

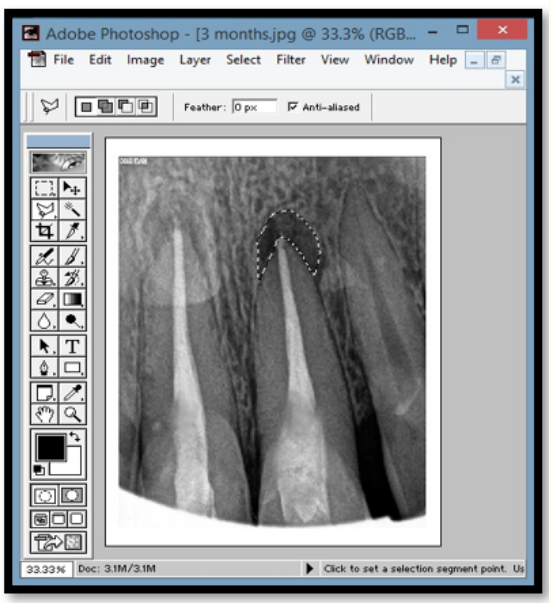

Figure 3: Morphometric area analysis, selection of the bone fill region.

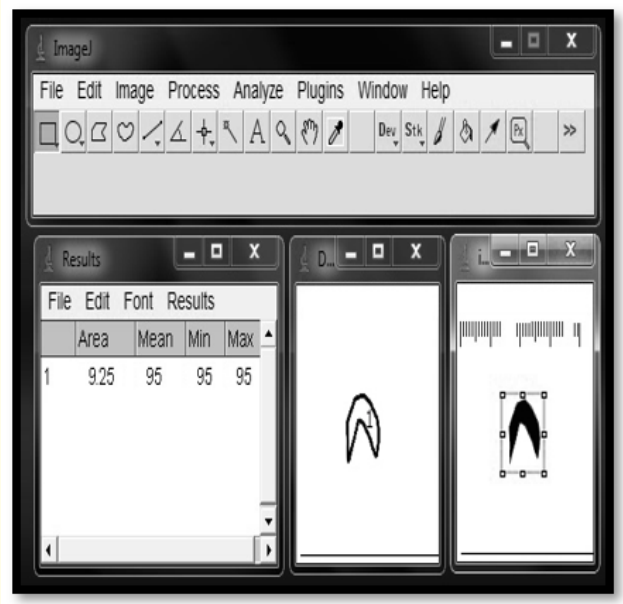

Figure 4: Layer area calculation in Image J® software.

Results

Healing was evaluated clinically and radiographically for a period of 6 months after surgery. Table 1 and 2 shows the comparison of change from baseline using One-Way ANOVA with Post-Hoc Turkey HSD. Significant P value is seen between Group 2 Vs Group 3. In Group 1 Vs Group 2 change is more compared to Group 1 Vs Group 3 but not statistically significant. Intra group comparison between Test Vs control, in all these Groups paired 't' test was done which showed more significance difference at the end of 6 months with group 3 i.e. 0.001. On comparing change between Test Vs Control from baseline to 3 months and from baseline to 6 months, showed more change with Group 3 containing PRF and DFDBA followed by Group 1 containing PRF and Group 3 containing DFDBA. On comparing percentage in bone formation from baseline to 6 months in Test vs Control showed more percentage change with Group 3 test containing both PRF and DFDBA.

Time	Group 1 Mean ± SD		Group 2 Mean ± SD		Group 3 Mean ± SD	
	Test	Control	Test	Control	Test	Control
Baseline to 3 months	3.81 ± 1.09	2.87 ± 0.95	2.30 ± 1.29	1.89 ± 0.77	4.84 ± 1.67	3.01 ± 1.67
Baseline to 6 months	7.59 ± 1.61	6.05 ± 1.61	4.83 ± 2.06	4.05 ± 1.02	8.08 ± 3.02	5.90 ± 1.14
% age change Baseline to 6 months	76.96 **± 15.65**	60.66 ± 9.06	71.23 **±16.34**	55.94 **± 19.07**	89.75 ± 9.49	65.10 **± 15.76**

Table 1*: Comparing change from baseline in each group.*

Time	Group 1 vs 2 P value#		Group 1 vs 3 P value#		Group 2 vs 3 P value#	
	Test	Control	Test	Control	Test	Control
Baseline to 3 months	0.096	0.251	0.312	0.972	0.004*	0.172
Baseline to 6 months	0.065	0.014*	0.905	0.971	0.027*	0.023*
% change Baseline to 6 months	0.702	0.811	0.192	0.830	0.041*	0.464

Table 2: *Comparing change from baseline between the groups.*
*#One-Way ANOVA with Post-Hoc Tukey HSD; *p < 0.05: Significant.*

Discussion

The success of endodontic therapy depends on complete periapical repair and regeneration. The healing of hard and soft tissues is mediated by wide range of intra and extra cellular events that are regulated by signaling proteins [6]. Periapical surgery includes the curettage of all periapical soft tissues and sometimes application of different biomaterials to enhance new bone formation at a defective site. PRF was first developed in France by Choukroun., *et al*. It has been referred to as a second-generation platelet concentrate, which has been shown to have several advantages over traditionally prepared PRP. Its chief advantages include ease of preparation and lack of biochemical handling. The ability of PRF to polymerize and form three-dimensional supramolecular assemblies with entrapped platelet cytokines (intrinsic cytokines) seem to be a fundamental advantage for tissue engineering [7]. PRF has plastic and soft tissue adhesive properties, this autologous material is highly bioactive. A single PRF membrane slowly releases high amounts of growth factors such as transforming growth factor, platelet derived growth factor, vascular endothelial growth factor and matrix proteins such as thrombospondin-1 (TSP-1) which plays significant role in the coagulation pathways and modulates cell matrix interactions [8]. Ozdemir., *et al.* [9] conducted an animal study, when used in conjunction with titanium barriers, PRF use can increase the quality of newly formed bone and enhance the rate

of bone formation due to concentration of growth factors. Yang., *et al.* [10] also conducted an animal study and demonstrated that using autogenic cell transplantation in a porcine model, that dental bud cells (DBCs) seeded into fibrin glue-PRF could regenerate a complete tooth. Hence defects treated with PRF In Group 1 showed significant improvements in all clinical parameters compared with control and Group 2.

DFDBA demonstrates osteoinductive property through bone morphogenic proteins which is supplied in a sterile plastic syringe of 0.5 cc quantities. Reasons for selection of DFDBA as graft material are its documented studies to induce bone formation in ectopic sites and its ability to produce significantly greater defect fill than that seen in open flap debridement alone, and its ability to enhance regeneration [11]. Ashish Agarwal [12] conducted a study on infrabony defects in split mouth technique using PRP and DFDBA in one defect and DFDBA alone in another defect. After 12 months follow up they observed significant increase in clinical and radiographic parameters in test group containing combination of DFDBA and PRP. Our study results are similar to these studies.

Size of lesions also effect prognosis of surgery. All cases in this study are below 1 - 15 mm. Healing rates is significantly higher for teeth with smaller rather than larger preoperative lesions [13].

A combination of PRF with DFDBA demonstrated better results in periapical bone formation compared to DFDBA alone. This result may be attributed to beneficial effects of PRF. It 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 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. DFDBA enhances the effects of PRF by maintain the space for tissue regeneration to occur as well as osteoconductive effect in periapical defects. Such a mechanism might explain the clinically observed soft tissue and hard tissue healing properties of combination of PRF and DFDBA. However, histological studies are needed to establish the exact nature of this.

Conclusion

In the course of this investigation regeneration techniques were found to be superior to normal healing at the two observation periods. However, differences between the control and experimental groups were more evident after a period 6 months. Group III containing combination of PRF and DFDBA showed better results than other two groups containing PRF and DFDBA individually, but further long-term follow up as well as histological nature of newly formed tissues by either treatment remains to be elucidated.

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