Clinical effectiveness of autologous platelet rich fibrin in the management of infrabony periodontal defects

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Abstract

Background: This interventional controlled clinical trial with split mouth design compares the clinical effectiveness of autologous platelet rich fibrin with open flap debridement in the management of infrabony periodontal defects.

Methods: Fifteen patients with paired contralateral infrabony defects were treated with open flap debridement and autologous platelet rich fibrin (experimental group) or open flap debridement alone (control group). The changes in probing pocket depth, clinical attachment level, and radiographic defect depth were evaluated. Patient perception regarding pain and discomfort following the procedures and early soft tissue healing responses were assessed by visual analog scales, scored 7 days after the surgical procedures. Final reevaluation was done 1 year after surgery.

Results: Baseline clinical and radiographic measurements were comparable between the groups. Reevaluation at 1 year revealed that both treatment modalities resulted in a significant decrease in probing pocket depth, gain in clinical attachment and radiographic bone fill of the defects compared to baseline. Postoperative differences observed between the two groups were 2.27 ± 0.29 mm (P < 0.001) for probing pocket depth, 3.33 ± 0.35 mm (P < 0.001) for clinical attachment level and 1.29 ± 0.32 mm (P < 0.001) for radiographic infrabony defect depth reduction, all in favor of the experimental group. Patient preference was greater and early healing response better for the experimental group as assessed by the visual analog scores.

Conclusion: Within the limitations of this study it can be concluded that use of platelet rich fibrin is more effective than open flap debridement alone in the management of infrabony periodontal defects.

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The ultimate goal of periodontal therapy is the regeneration of lost tissues. Periodontal regeneration involves the formation of alveolar bone, cementum, and a new functional periodontal ligament [1]. For periodontal regeneration to occur, a number of biologic events; including cell migration, adherence, multiplication, and differentiation, need to occur in a well-orchestrated sequence [2].

For many years, research has attempted to use biologically active molecules to achieve periodontal regeneration. Polypeptide growth factors (PGFs) are biologic mediators that have the ability to regulate cell multiplication, migration, and differentiation. Several PGFs have been identified in human periodontal tissues [3]. Of all known PGFs, platelet-derived growth factor (PDGF) was shown to exert a favorable effect on periodontal regeneration as measured by gain in clinical attachment and radiographic defect fill in humans [3,4].

Though the use of growth factors has shown tremendous promise in periodontal regenerative approaches, the routine use of these growth factors in everyday clinical practice has not been achieved so far. One of the major challenges pertaining to the use of growth factors was the non-availability of an ideal carrier [5]. Platelet-rich plasma (PRP) was first introduced as a delivery system for growth factors in 1998 by Marx et al. [6]. Though diverse clinical reports are available with regard to advantages of adjunctive use of platelet concentrates to periodontal surgical procedures [7,8], the most recent systematic review and meta-analysis has concluded that platelet concentrates may exert a positive adjunctive effect when used for the treatment of infrabony defects [9].

A recent innovation in the field of dentistry is the development of autologous platelet rich fibrin matrix (PRFm) as a growth factor delivery system. Platelet rich fibrin is a second generation platelet concentrate developed by Choukroun et al. [10] in 2005. It is nothing but centrifuged blood without any addition and avoids any kind of biochemical handling of blood. The combined properties of fibrin, platelets, leukocytes, growth factors and cytokines makes platelet rich fibrin a healing biologic material [11] with tremendous potential for bone and soft tissue regeneration. The available data are limited, and further investigation is required to assess the regenerative potential of platelet rich fibrin, which led us to examine the hypothesis of an enhanced regenerative outcome of PRFm in infrabony periodontal defects.

The aim of this interventional controlled clinical trial was to assess the clinical effectiveness of PRFm to bring about periodontal regeneration by comparing it with conventional open flap debridement in periodontal infrabony defects. This study also aimed to assess the patient perception and preference for these two surgical techniques considering pain and discomfort during the first week of surgery and the differences in early healing response by means of visual analog scales.

2. Materials and methods

This controlled clinical trial with a split mouth design was conducted in the department of Periodontics, Government Dental College, Kozhikode, Kerala, India from September 2009 to October 2010. The study consisted of an experimental group which was treated by placement of platelet rich fibrin following open flap debridement (OFD+PRFm) and a control group treated by open flap debridement (OFD) alone. Clinical and radiographic parameters were reevaluated after 1 year.

2.1. Sample size

The ideal sample size to assure adequate power for this clinical trial was calculated as described by Chan [12]. It was determined that 11 defects per group would be necessary to provide 80% power with \( \alpha \) of 0.05.

Fifteen systemically healthy, non-smoking subjects were selected for the study. Prior to initiating this study, the patients were informed of the purpose and design of this clinical trial and were required to sign an informed consent. The study design and consent form were approved by the Institutional ethics committee, Government Dental College, Kozhikode in accordance with the Helsinki Declaration of 1975 as revised in 2000.

The criteria for inclusion of subjects in this study were individuals who:

1. had paired, contralateral interproximal infrabony defect with a probing pocket depth (PD) \( \geq 6 \text{ mm} \), clinical attachment level (CAL) loss \( \geq 5 \text{ mm} \), and an osseous defect depth estimated from radiographic evaluation (IBD) as \( \geq 4 \text{ mm} \);
2. were systemically healthy without a history of allergies; and
3. had at least 2 mm of keratinized gingiva on the facial aspect of the selected tooth.
The following patients were excluded from the study:

1. Hematological or immunological disorders.
2. Pregnancy or lactation.
3. Smoking or the use of other tobacco products.
4. Those taking drugs known to interfere with wound healing.
5. Had used antibiotics within the previous 1 year;
6. Had been treated for periodontitis during the previous 2 years.
7. Those with unacceptable oral hygiene (plaque index (PI) > 2) after the reevaluation of phase I therapy.
8. Were not willing to sign an informed consent.

2.1.1. Presurgical therapy
Prior to the surgery, after careful instructions on proper oral hygiene measures full mouth scaling and root planing procedures were performed under local anesthesia. Six to eight weeks following phase I therapy, periodontal evaluation was performed to confirm the suitability of the sites for this study and baseline data was recorded. The sites were divided into experimental and control groups at the time of periodontal surgery. Either right sided or maxillary defects were operated first and whether the site belonged to experimental or control group was determined by a simple lottery method by the toss of a coin.

2.1.2. Pro forma
A detailed questionnaire was used to record demographic data, clinical and radiographic parameters.

2.1.3. Clinical parameters
A clinical examination was performed by a single examiner (NS) who was masked to the treatment group to which a patient was assigned, at baseline and 1 year after the surgical procedure. The clinical parameters assessed included probing depth (PD), recession/enlargement (REC), and clinical attachment level (CAL). Patient oral hygiene status and gingival inflammation were evaluated using plaque index (PI) [13] and Modified Gingival Index [14] (MGI) respectively.

2.1.4. Radiographic examination
Standardized reproducible radiographs using paralleling cone technique with positioning aids were taken at each experimental and control site at baseline and 1 year after surgery. All radiographs were evaluated by a single examiner (RJ) who was masked to the treatment group to which a patient was assigned and also to whether the radiograph was taken at baseline or reevaluation. All radiographs were superimposed on a standardized transparent calibration sheet and measurements were made. Radiographic infrabony defect depth (IBD) was assessed using the method described by Cardaropoli and Leonhard [15]. The vertical dimension between the projection of the bone crest on the root surface (BCP) and the most coronal level along the root surface where the periodontal ligament space was considered to have a normal width (BoBD—base of bone defect) was measured and designated as infrabony defect depth (IBD = BCP – BoBD). The distance from the crest of remaining alveolar bone to the cementoenamel junction (CEJ) was also recorded (CEJ-BC) (Fig. 1).

2.1.5. Treatment procedures
All periodontal surgical procedures were performed by a single operator (AR). Standard surgical procedures for experimental and control sites were performed as follows. After local anesthesia, crevicular incisions were made and full-thickness mucoperiosteal flaps were elevated. Vertical releasing incisions were performed only if necessary for better access or to achieve more favorable closure of the surgical site. Meticulous defect debridement and root planing were carried out to remove sub gingival plaque, calculus, inflammatory granulation tissue, and pocket epithelium.

2.1.6. Preparation of platelet rich fibrin matrix (PRFm)
10 ml blood was drawn by venipuncture of the right antecubital vein. Blood was collected in sterile glass test tubes without any anticoagulants and immediately centrifuged on a table top centrifuge (KW-70, Almicro™ Instruments, Ambala Cantt., Haryana, India) at 3000 rpm for 10 min. This resulted in the separation of three basic fractions because of differential densities: the bottom red blood cells (RBCs), middle platelet rich fibrin (PRFm), and the top layer of platelet-poor plasma (PPP). PPP was aspirated and discarded and the PRFm was separated from underlying RBCs by the

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Fig. 1 – Illustration showing radiographic measurements performed. BC—bone crest level. BCP—projection of the bone crest on the root surface, BoBD—base of bone defect, IBD—distance from BCP to BoBD.
use of sterile stainless steel scissors. The PRFm was immediately placed into the infrabony osseous defects in the experimental group. Surgical flaps were repositioned to their presurgical level and sutured with 4-0 silk sutures achieving primary closure. Periodontal packs were placed to cover the surgical areas. Control sites were treated in every way similar to experimental sites except for the preparation and placement of PRFm.

2.1.7. Postsurgical care

Postoperative care included systemic administration of amoxicillin, 500 mg, every 8 h for 5 days, paracetamol 500 mg every 8 h for 3 days and 0.2% chlorhexidine digluco- nate rinse three times daily for 6 weeks. Sutures were removed 1 week post-surgery.

A visual analog scale (VAS1) was used to assess the patient experience with the two treatment modalities. At 1 week after the procedure the patient was asked to assign scores for the surgical procedure with a minimum score of 0 and a maximum score of 10 taking into consideration: (1) pain during the first week after surgery and duration for which the pain lasted; (2) redness and discomfort during the first week after surgery; and (3) overall perception of the surgical procedure and patient opinion regarding the procedure. Study subjects assigned maximum scores to the surgical procedure of their preference. Thus higher scores indicated higher patient acceptance and minimum scores to the surgical procedure.

2.1.8. Maintenance phase

After suture removal, mechanical plaque control using the roll tooth brushing technique was resumed at the surgically treated sites. The patients were recalled once a month up to 1 year post-surgery for oral hygiene reinforcement and prophylaxis. All clinical and radiographic measurements were rerecorded at the end of 1 year.

For the visual analog scores, the frequency with which each score occurred was recorded for the experimental and control groups. Intra- and intergroup comparisons were made using the chi-square test.

3. Observations and results

The study group consisted of 15 patients with a mean age of 29.47 ± 7.65 years (range 17–44 years). There were 9 female and 6 male patients in the study group. The mean plaque index scores at baseline was 1.24 ± 0.25 and the mean modified gingival index scores were 1.27 ± 0.34. Proper oral hygiene maintenance was ensured throughout the maintenance phase. All 15 patients completed the study. Defects in the
experimental and control groups healed uneventfully. No cases of flap dehiscence or infection were detected.

Both groups were comparable at baseline with respect to probing depth (PD), clinical attachment level (CAL), gingival recession (REC) depth of the infrabony defects (IBD) and distance from the cementoenamel junction to bone crest (CEJ-BC) (Table 1).

Both the experimental group and the control group showed a significant gain in clinical attachment levels and probing depth reduction and reduction in infrabony defect depth at 1 year (Table 2). There was no significant gingival recession in the experimental group during the 1 year after the surgery. However there was a statistically significant gingival recession with a mean value of 1.13 ± 0.74 mm in the control group during the same time interval (Table 2).

Though both groups achieved statistically significant improvements in the clinical and radiographic parameters assessed it was found that the magnitude of improvements in these parameters were significantly higher for the experimental group (Table 3).

Experimental sites presented with greater mean clinical attachment gain with a mean difference of 3.36 ± 0.38 mm between the groups (P < 0.000). The postoperative differences in probing depth between the two groups were found to be 2.29 ± 0.3 mm (P < 0.000) in favor of the experimental sites (Table 3). There, was a statistically significant difference of 1.2 ± 0.2 mm in levels of gingival recession between the two groups indicating a lesser mean gingival recession in the experimental group (Table 3).

Experimental sites presented with a greater amount of reduction in infrabony defect depth with a mean difference in reduction of 1.29 ± 0.32 mm (P < 0.000) (Table 4). There, was no significant crestal bone resorption (CEJ-BC) in either of the groups (P = 0.541) (Table 3).

For VAS1, in the experimental group the most commonly recorded scores were 7 and 8 (55%). In the control group scores 6 and 5 (67%) were most common. This difference in frequency of scores was found to be statistically significant between the groups (Table 4).

In VAS2 assessing the early healing soft tissue changes; in the experimental group the score 1 (33%) and score 2 (47%) were most common. In the control group there was no site with a score of 1. Scores 2 (53%) and 3 (47%) were most often recorded. There was a statistically significant difference in the frequency of scores between these two groups (Table 5).

### Table 3 – Comparison of Mean changes obtained for clinical and radiographic parameters between the two groups at reevaluation (Mann–Whitney test).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Experimental (Mean ± SD)</th>
<th>Control (Mean ± SD)</th>
<th>change between groups</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>PD change (mm)</td>
<td>4.67 ± 0.90</td>
<td>2.40 ± 0.63</td>
<td>2.27 ± 0.29</td>
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</tr>
<tr>
<td>CAL change (mm)</td>
<td>4.73 ± 0.88</td>
<td>1.40 ± 1.06</td>
<td>3.33 ± 0.35</td>
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<tr>
<td>REC change (mm)</td>
<td>−0.07 ± 0.26</td>
<td>1.13 ± 0.74</td>
<td>1.20 ± 0.20</td>
<td>0.000</td>
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<tr>
<td>CEJ-BC change (mm)</td>
<td>0.07 ± 0.62</td>
<td>0.14 ± 0.54</td>
<td>0.21 ± 0.21</td>
<td>0.541</td>
</tr>
<tr>
<td>IBD change (mm)</td>
<td>1.93 ± 1.07</td>
<td>0.64 ± 0.50</td>
<td>1.29 ± 0.32</td>
<td>0.001</td>
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</table>

### Table 4 – Patient perception and acceptance between the two groups (VAS1).

<table>
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<th>P value</th>
</tr>
</thead>
<tbody>
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<td>3</td>
<td>0.024</td>
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<td>5</td>
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<td>4</td>
<td></td>
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<td>2</td>
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<tr>
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<tr>
<td>Total</td>
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### Table 5 – Assessment of early healing response (VAS2).

<table>
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<th>P-value</th>
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<tbody>
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<td>2</td>
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<tr>
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<tr>
<td>Total</td>
<td>15</td>
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</table>

4. Discussion

For the study, 30 infrabony sites in 15 patients were selected using a split-mouth design. Though recent evidences suggest both parallel and split mouth designs to be equally effective [9], the split mouth design was chosen as this permits better assessment of how the same host responds to two different treatment modalities.

The sample size was enough to ensure 80% power for the study. The sample size selected for this study was comparable to other interventional clinical trials in periodontal literature. Randomization was not attempted in this clinical trial because in split mouth design when one site was randomly assigned as either experimental or control the other site automatically selected itself to the remaining group. True randomization was hence possible for only for half the sites.

Patient blinding regarding the type of therapy was not possible in this trial because of the procedures of blood collection and preparation of platelet rich fibrin associated with the experimental sites. However the investigators
performing clinical and radiographic evaluations were masked of the treatment group as to which the study site belongs.

Platelet rich fibrin alone was used in the experimental group and not as an adjunct to other regenerative approaches like bone replacement grafts or guided tissue regeneration in the present study. There has been conflicting reports regarding the use of platelet concentrates along with bone replacement grafts. Even though some studies claim a superior clinical effectiveness for the combination [16], reports claiming no added advantages for the combination are also available [17]. The recent metaanalysis [9] states that combination of platelet concentrate with guided tissue regeneration (GTR) masks the true effectiveness of platelet concentrates.

In the present study platelet rich fibrin was used rather than the more extensively studied platelet rich plasma as it offers several advantages like ease of preparation, no biochemical handling of blood or use of any gelling agent like calcium chloride and no risks associated with the use of bovine thrombin. As it is a completely autologous material it is highly cost effective.

The success of this technique entirely depends on the speed of blood collection and immediate centrifugation [10]. In order to obtain a clinically usable platelet rich fibrin clot, in the present study a chair side centrifuge was used and it was ensured that the freshly drawn blood was immediately transferred to the centrifuge without any delay to prevent dehydration.

The results of this clinical trial indicate a positive effect for the use of platelet rich fibrin in the management of infrabony periodontal defects in terms of improvement in clinical and radiographic parameters.

At reevaluation one interesting finding in the present study was the absence of post-operative increase in gingival recession in the experimental group as compared to controls. Moreover the experimental group shows a mild decrease in preoperative recession levels (0.07 ± 0.62 mm). Even though this value is of no clinical significance; this calls for more clinical studies using platelet rich fibrin in the management of gingival recession.

The improvement in clinical parameters and better bone fill in the experimental group are suggestive of the effectiveness of platelet rich fibrin in regenerative periodontal therapy. These results may be attributed to the contents of the platelet rich fibrin clot namely fibrin, platelets, leukocytes, growth factors and cytokines.

The fibrin matrix supporting the PRFm clot constitutes the determining element responsible for the therapeutic potential of platelet rich fibrin [11]. The fibrin matrix plays important role in four highly specific aspects of healing: angiogenesis, immune control, harnessing the circulating stem cells, and wound protection by epithelial cover [11].

The angiogenesis property of fibrin matrix is explained by the 3-dimensional structure of the fibrin gel and by the simultaneous action of cytokines trapped in the meshes. During hemostasis and healing, the fibrin clot traps the circulating stem cells and allows the vascular and tissue restoration. An important phase of angiogenesis is αvβ3 integrin expression by endothelial cells, allowing the cells to bind to fibrin, fibronectin, and vitronectin. Regulation of this integrin expression could be brought on by fibrin itself [18].

Among the growth factors contained in the platelet rich fibrin clot, platelet-derived growth factors (PDGF), Insulin-like growth factors (IGF) and transforming growth factor β (TGF-β) play the most important roles. PDGF-α and -β receptors are expressed in regenerating periodontal soft and hard tissues. PDGF initiates periodontal ligament cell chemotaxis, mitogenesis and matrix synthesis. Application of PDGF alone or in combination with IGF-1 results in partial repair of periodontal tissues [19].

In the present study we observed a significant bone fill in the experimental group. Direct interactions between fibrin and osseous cells during healing are insufficiently documented [11]. On the other hand, numerous animal studies deal with the fibrin effect on osseous healing. The results are contradictory; osseous healing is either improved or remains unchanged [20]. Growth factors contained in the platelet rich fibrin clot could have contributed to the radiographic bone fill observed in the present study. PDGF has been shown to have a significant regenerative impact on FDL cells and osteoblasts [21]. It has also been reported that PRFm induces a significant and continuous stimulation of proliferation in all cell types of the periodontium except epithelial cells [22]. PRFm stimulates human bone mesenchymal cell proliferation and differentiation [23].

Our results in the experimental group were compared to those studies using platelet rich plasma alone as periodontal regenerative approach [16,17]. Our results are in accordance with these studies in terms of changes in clinical attachment levels, probing depths, and radiographic bone fill. However the magnitude of change in CAL and PD at reevaluation is much higher in the present study as compared to studies using PRP alone.

It has been reported that the CAL gain after conventional or regenerative periodontal treatment was dependent on the initial PD; that is, deeper the initial PD, the greater the PD reduction and the CAL gain [24]. This is significant considering that the baseline levels of probing pocket depth and Clinical attachment levels in the present study were comparable to studies that used PRP [16,17]. Markou et al. [17] reported a mean improvement in PD of 3.92 ± 1.1 mm, CAL of 3.08 ± 0.95 mm 1 year after periodontal surgery. Tunc ilgenli et al. [16] reported a mean improvement in PD of 2.1 ± 0.5 mm, CAL of 1.5 ± 0.7 mm and a radiographic reduction of infrabony defect depth of 0.6 ± 1.2 mm 18 months after surgery. In contrast, the present study reports a mean change in PD of 4.67 ± 0.90 mm, CAL of 4.73 ± 0.88 mm and a radiographic reduction in infrabony defect depth of 1.93 ± 1.07 mm at 1 year after surgery.

The reason for the improved results with platelet rich fibrin may be attributed to the difference in structure between PRP and PRFm [10] and their growth factor content. PRP uses a bovine thrombin and calcium chloride resulting in sudden fibrin polymerization. PRFm has the characteristic of polymerizing naturally and slowly under physiologic concentrations of autologous thrombin. This difference in polymerization results in two different biochemical architectures for the resulting product: Condensed tetra molecular or bilateral junctions in PRP and connected trimolecular or equilateral junctions in PRFm [25].
Bilateral junctions are constituted with strong thrombin concentrations and allow the thickening of fibrin polymers; this leads to the constitution of a rigid network, not very favorable to cytokine enmeshment and cellular migration. In contrast equilateral junctions allow the establishment of a fine and flexible fibrin network able to support cytokines enmeshment and cellular migration. Moreover, this 3-dimensional organization will give great elasticity to the fibrin matrix.

There are wide variations in quantity of growth factor released from platelet concentrates prepared using different preparation protocols. A study by Gassling [26] assessed Growth factor release from PRP and platelet rich fibrin (PRF) and found that after 10 days the amount of growth factors released from PRP is higher than that from PRF. However in a comparative study He et al. [27] concluded that PRF released autologous growth factors gradually and expressed stronger and more durable effect on proliferation and differentiation of rat osteoblasts than PRP in vitro.

Our study also evaluated the patient perception with respect to the two surgical procedures using a visual analog scale. Majority of patients reported a preference for regenerative surgery with PRFm. It has been proposed that PRFm is a healing biomaterial that accelerates wound closure and mucosal healing, with a significant diminution of pain and discomfort, due to fibrin bandage and growth factor release [11]. However a Hawthorne effect could have also played a part in these results as the patient could not be blinded to the procedures.

A second visual analog scale was used to assess the early wound healing. The experimental group had significantly lower scores indicating that PRFm indeed accelerates early wound healing. Degranulation of leukocytes in the PRFm could release cytokines into the fibrin clot. The major cytokines reported to be present in PRFm are proinflammatory cytokines like interleukin 1β (IL1 β), interleukin 6(IL 6), tumor necrosis factor α (TNF α) and anti-inflammatory cytokines interleukin 4(IL 4). SoPRFm clot could be considered as an immune organizing node and its defense capacities against infections would be quite significant [28]. This could have contributed to the better healing responses and enhanced patient comfort.

Whether the damaged tissues heal by regeneration or repair following any periodontal regenerative approach depends upon two crucial factors: the availability of cell type(s) needed; and the presence or absence of cues and signals necessary to recruit and stimulate these cells [29]. The use of platelet rich fibrin provides a convenient approach by which the presence of both these factors can be expected at the surgical sites. Thus the added advantages of fibrin and the sustained release of growth factors present in the platelet rich fibrin matrix could be responsible for the superior results observed in the experimental group of our study. Future researches focusing on clinical trials and histological evaluations are necessary to further assess the periodontal regenerative potential of platelet rich fibrin.

The limitations associated with this controlled interventional clinical trial are the inability to do true randomization and assessment of periodontal regeneration through assessment of clinical and radiographic parameters alone.

5. Conclusions

Within the limitations of this study it can be concluded:

1. Use of platelet rich fibrin significantly improved the clinical and radiographic parameters that were assessed in this study.
2. Platelet rich fibrin significantly reduced the postoperative pain and discomfort after periodontal surgery and significantly accelerated periodontal wound healing.

It remains to be seen whether the adjunctive use of other regenerative approaches along with platelet rich fibrin increases its clinical effectiveness or masks its true regenerative potential. Multicenter trials with large sample size and longer follow up time are required to further assess the regenerative potential of platelet rich fibrin.

REFERENCES


