Effect of Platelet-rich Fibrin on Healing of Apicomarginal Defects: A Randomized Controlled Trial

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Abstract

Introduction: The purpose of this prospective, randomized controlled trial was to evaluate the healing outcomes of platelet-rich fibrin (PRF) in periapical surgeries involving apicomarginal defects and to compare these results with surgeries not using any guided tissue regeneration techniques. Methods: Thirty patients with suppurative chronic apical periodontitis and apicomarginal communication were randomly assigned to either the PRF or the control group. Clinical and radiographic parameters including pocket depth (PD), clinical attachment level, gingival marginal position, size of periapical lesion, and percentage reduction of the periapical radiolucency were recorded at baseline and at an interval of 3 months for a period of 12 months. Results: The overall success rate was 83.33%, with a success rate of 86.66% (13 of 15 teeth) for PRF group and 80% (12 of 15 teeth) for control group. Both the groups exhibited a significant reduction in PD, clinical attachment level, gingival marginal position, and size of periapical lesion at 12-month period. No significant differences were observed between the 2 groups for these parameters except PD, which showed a statistically significant reduction in the PRF group (P < .05). Conclusions: The adjunctive use of regenerative techniques may not promote healing of apicomarginal defects of endodontic origin. (J Endod 2015;41:985–991)

Key Words

Apicomarginal communication, guided tissue regeneration, periradicular surgery, platelet rich fibrin, success rate

Apicomarginal defects are periradicular lesions accompanied by a periodontal breakdown with a reported incidence of 3.56% (1) to 20% (2). These defects are associated with relatively lower success rates after endodontic surgery (3, 4). Apical migration of junctional epithelium has been attributed to be an important factor in lower success of apical surgeries for these defects (5).

Recently, the use of autologous products has been preferred over the traditional practice of using guided tissue regeneration (GTR) barrier membranes for the treatment of apicomarginal defects (6). Platelet-rich plasma (PRP), a first-generation platelet concentrate, has emerged as an alternative to GTR membrane, with a comparable success rate (7, 8). Goyal et al (9) reported a significant percentage reduction of the periapical rarefaction, pocket depth (PD) reduction, and gain of attachment level by using PRP for treating apicomarginal defects. However, the need for biomodification with an anticoagulant, time taking procedure, and use of bovine thrombin/calcium chloride for activation are some of the disadvantages that limit its use (10).

Recently developed second-generation platelet concentrate, platelet-rich fibrin (PRF), is being suggested as a better alternative to PRP. PRF is a strictly autologous, slowly polymerized, high-density cicatricial fibrin network with an intimate assembly of cytokines, glycan chains, and structural glycoproteins (11). PRF acts as an immune regulation node and promotes wound healing via various growth factors such as platelet-derived growth factor-β, transforming growth factor-β, vascular endothelial growth factor, and insulin-like growth factor-I and inflammatory cytokines such as interleukin 1β, interleukin 6, interleukin 4, and tumor necrosis factor-α (11). Compared with PRP, PRF has fewer chances of cytotoxicity, immunogenicity, and cross-reactivity. It can be instantly obtained as a fibrin membrane with a rather simplified and inexpensive procedure without the need of any biochemical manipulation (12). PRF releases a relatively constant concentration of growth factors and matrix proteins (thrombospondin-1, fibronectin, vitronectin) during a period of 7 days (13) and is linked to enhanced expression of alkaline phosphatase (highest at day 14), thereby exerting a stronger long-term stimulatory effect on osteoblasts (14) in contrast to PRP.

Application of PRF in plastic surgery (15) and oral and maxillofacial surgeries (16) has shown promising results. Moreover, PRF as a regenerative material has been found to be beneficial in the treatment of intrabony and furcation defects (17–19). However, no study has investigated the efficacy of PRF membrane in endodontic surgeries involving apicomarginal defects. Therefore, the purpose of this prospective, double-blind, randomized controlled trial was to evaluate the healing of apicomarginal defects after endodontic surgery by using PRF and also to compare the healing outcomes of PRF with surgeries not using any GTR technique in such lesions.

Materials and Methods

The study was conducted in the Department of Conservative Dentistry and Endodontics, Post Graduate Institute of Dental Sciences, Rohtak, India, and subjects were recruited between May 2011 and December 2013. The study protocol was approved by the Ethical Committee (ECR/495/Inst/HRI/2013), Post Graduate Institute of Dental Sciences, Rohtak, India and accords well with the principles embodied in the Declaration of Helsinki 1975, as revised in 2000.
Subject Enrollment and Inclusion/Exclusion Criteria

Subjects were selected from the pool of patients referred for periradicular surgery with diagnosis of suppurative chronic apical periodontitis and apicomarginal communication (Fig. 1). Eligibility criteria included patients with a deep narrow pocket with probing depth >6 mm confined to buccal aspect of the root, negative response to vitality tests, radiographic evidence of radiolucency, failed previous root canal treatment and retreatment at least 1 year previously, previous surgery with unresolved bony lesion, recurrent episodes of purulent discharge, and adequate final restoration with no clinical evidence of coronal leakage. Chronic generalized periodontitis, any systemic disease contraindicating oral surgical procedures, evidence of root fracture, and resorptive processes involving more than apical third of the root were regarded as exclusion criteria. Informed consent was acquired from all participants after carefully explaining the possible risks and benefits.

Sample Size Calculation

The anticipated success rate for PRF was expected to be 80% (9), and that of control group was expected to be 27% (3). A power calculation that was based on the data suggested that a sample size with 14 patients in each group would have an 80% power of detecting differences between treatments (alpha at the 5% level and effect size of 1.06).

Preoperative Procedures and Primary Outcome Measurements

All eligible patients were given careful instructions regarding proper oral hygiene measures and were subjected to a series of professional plaque control. Occlusal adjustments were done if a traumatic occlusion was noticed. Patients were recalled for baseline examination 1 week after this initial therapy. All preoperative clinical periodontal measurements including PD, clinical attachment level (CAL), and gingival margin position (GMP) were recorded. As a reference for CAL and GMP, the cementoenamel junction or the apical borders of restorations when cementoenamel junction was not visible were used. Clinical parameters were measured circumferentially around the tooth (to the nearest millimeter) by using William’s periodontal probe. Defects involving the proximal root surface were excluded. Only the deepest measurements were recorded.

Radiographic examination was performed at 0, 3, 6, 9, and 12 months by using Rinn (XCP Instruments, Elgin, IL) paralleling technique and digitized by using Kodak RVG 6000 (Kodak Digital Radiography System, Pt Husada Intra Care, Banten, Indonesia). A customized jig was prepared by using addition of silicone putty and preserved for future radiographic references.

Randomization

Randomization was developed to eliminate any bias on the part of the investigator and to balance the number of patients between the

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**Figure 1.** A CONSORT diagram showing the flow of participants through each stage of trial.
surgery types. By using a 50:50 randomization allocation ratio, a research associate (S.K.) created opaque envelopes that contained concealed assignment codes assigned sequentially to eligible patients. Patients’ codes were broken once the apicomarginal defect was confirmed after raising the mucoperiosteal flap.

**Surgical Techniques**

With the exception of incisions, flap elevation, and suturing, all surgical procedures were performed under an operating microscope (Moller denta 300; Haag Streit International, Koniz, Switzerland) by a single operator (M.D.) at 2–24× magnification.

The operator was blinded to which group the patient had been placed until the time of surgery. Patients were anesthetized by using 2% lidocaine with 1:80,000 epinephrine (Xylocaine; Astra Zeneca Pharma, Bangalore, India). A full-thickness mucoperiosteal flap was reflected, and an osteotomy was performed with round bur under copious saline irrigation, followed by debridement (periradicular curettage–enucleation) of the bony lesion. The periosteal surface of flap was frequently irrigated to prevent dehydration. For additional hemostasis, cotton pellets soaked in 0.1% epinephrine (Jackson Lab Ltd, Punjab, India) were applied topically. Approximately 3-mm root tip with 0° to 10° bevel angle was sectioned with a cylindrical surgical carbide bur under copious water-spray. Root-end preparations of 3-mm depth were prepared by using S12-7D ultrasonic retrotips (Satelec, Paris, France) driven by a piezoelectric ultrasonic unit (P5 Booster, Suprasson Neutron; Acteon Inc, Mt Laurel, NJ) at medium power setting. The resected root surfaces were stained with methylene blue and inspected with micro mirrors (Hu Friedy, Chicago, IL) under 20–24× magnification. Any significant anatomic details and the cleanliness of the root-end preparation were carefully examined. Anatomic irregularities, if identified, were treated with the ultrasonic instruments. Root-end preparations were rinsed and dried with paper points. Mineral trioxide aggregate (Pro Root; Retroplast Trading, Rorvig, Denmark) was used as root-end filling material, and its adaptation to the canal walls was confirmed clinically at high magnification as well as radiographically.

**PRF Preparation**

PRF was prepared during surgery immediately before flap repositioning as described by Choukroun et al (20). The required amount of intravenous blood was collected in a 10-mL sterile Vacutainer without anticoagulant and centrifuged immediately by using a tabletop centrifugation machine (Remi Elektrotechnik Limited, Mumbai, India) at 3000 rpm for 10 minutes. PRF clot obtained in the middle of the tube was separated from red corpuscles base, preserving a 2-mm red blood cell layer by using sterile tweezers and scissors. The clot was then squeezed between 2 sheets of sterile cotton gauze to obtain a membrane, and the resultant fluid (PRF releasate) was discarded. PRF membrane was immediately placed (without storage) over the denuded root surface (Fig. 2), with the red blood cell end of the membrane facing the defect. Flap was repositioned and sutured by using 4-0 monofilament sutures. In the control group (Fig. 3), same surgical procedure was followed except that no PRF membrane was used.

An immediate postoperative radiograph was taken that served as a baseline reference. A postoperative mouthwash with 0.2% chlorhexidine gluconate (Hexidine; ICPA Health Products Ltd, Mumbai, India) was prescribed for plaque control. Sutures were removed 4–7 days postoperatively.

**Outcome Assessment**

Patients were recalled every 3 months for up to 12 months to evaluate any signs or symptoms of failure. Periodontal parameters (ie, PD, CAL, and GMP) were not measured until the last follow-up at the 12-month period. Follow-up radiographs were compared with postoperative radiographs, and periapical healing was defined
as complete, incomplete (scar tissue formation), uncertain (some reduction of former radiolucency), or unsatisfactory (no reduction or enlargement of former radiolucency) according to criteria described by Rud et al (21) and Molven et al (22). Results obtained were dichotomized into healed and non-healed categories. Cases classified under complete or incomplete healing with absence of clinical signs and/or symptoms were regarded as healed (successful), whereas those classified as uncertain or unsatisfactory were labeled as non-healed (failure).

**Procedure for Measuring Size of Periapical Lesion**

The radiolucent area in the apical third of the root, from the point at which the periodontal ligament showed continuous width, marked the periapical lesion. In case of difficulty in defining the periodontal ligament, the point where projection of the alveolar crest crossed the root surface was considered, and where multiple bony contours were encountered, the most external outline was considered. Two independent observers (S.T., P.S.) performed a blind radiographic assessment of the size of periapical lesion (SPL) in square millimeters. When discordant measurement data were reported, new examinations were repeated, and any further controversy was resolved by discussion. The digitized radiographs were divided into blocks by placing a grid on the image of periapical lesion by using CDR DICOM software (Schick CDR Technologies, Long Island City, NY). Dimension of each block measures 1 mm². The area of lesion was measured by counting the number of blocks occupying the lesion size. Only blocks with more than 50% of the area were considered, whereas those with less than 50% were eliminated.
TABLE 1. Demographic Data of Patients

<table>
<thead>
<tr>
<th>Groups</th>
<th>Men</th>
<th></th>
<th>Women</th>
<th></th>
<th>Age (y) (mean ± SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>%</td>
<td>n</td>
<td>%</td>
<td></td>
</tr>
<tr>
<td>PRF (n = 15)</td>
<td>10</td>
<td>66.67</td>
<td>5</td>
<td>33.33</td>
<td>27.93 ± 8.30</td>
</tr>
<tr>
<td>Control (n = 15)</td>
<td>9</td>
<td>60</td>
<td>6</td>
<td>40</td>
<td>25.67 ± 6.83</td>
</tr>
</tbody>
</table>

PRF, platelet-rich fibrin; SD, standard deviation.

Statistical Analysis

Results were expressed as the mean ± standard deviation. Results for each group showed a non-normal distribution (Kolmogorov-Smirnov test). Hence, the variables were analyzed by using non-parametric methods, Mann-Whitney and Wilcoxon rank sum tests for unpaired and paired data, respectively. Chi-square test was used to analyze the categorical data. The interobserver reliability was analyzed with the Cohen kappa analysis. Statistical tests were two-tailed and interpreted at the 5% significance level.

Results

Of the 52 patients initially recruited, a total of 30 patients aged 17–47 years fulfilled the eligibility criteria and were randomized into 2 groups. None of the participants were lost to follow-up. Table 1 presents the demographic characteristics for each group. For the PRF group, 10 men and 5 women ranging in age from 18 to 45 years participated in the study. For the control group, 9 men and 6 women ranging in age from 17 to 42 years participated. Majority of the participants in the study (63.33%) were men. Analysis of data revealed no statistical difference between groups with respect to age ($P = .624$) and gender ($P = .705$).

In each treatment group, PD, CAL, GMP, and SPL reduced significantly during the 12-month follow-up period (Table 2). GMP values became more negative by the end of 12 months, suggesting a recession from the original position. PRF group showed a statistically significant ($P = .046$) better improvement in PD than the control group, whereas there were no significant differences between the 2 groups for CAL or GMP (Table 3). Comparison of percentage reduction in SPL did not reveal significant differences at any time interval. For the PRF and the control groups, kappa values for the inter-rater agreement were 0.667 and 0.679, respectively, and the agreement was rated as good.

The categorization of the treatment outcome according to the clinical and radiographic parameters is shown in Table 4. The overall success rate was 83.33%, and the success rates in the PRF and the control groups were 86.66% (13 of 15 teeth) and 80% (12 of 15 teeth), respectively (Table 4). Statistical analysis of these success rates did not reveal any significant differences between the groups ($P = .624$). Two cases in the PRF group and 3 cases in the control group with a symmetrically placed rarefaction around the apex and decreased size of radiolucency were classified as uncertain healing.

Discussion

Apicomarginal defects, which are characterized by a localized complete loss of marginal bone and communicating with periodontal pocket, present a serious challenge to healing compared with the defects confined to periapical area. Use of a physical barrier has been suggested to prevent epithelial downgrowth over the denuded root surface (5, 23). However, with improved understanding of biological mechanisms mediating healing, the focus of attention is now shifting toward regenerative and reconstructive therapies (24). The present study evaluates the clinical usefulness of PRF in the treatment of apicomarginal defects.

We preferred PRF because, in addition to acting as a membrane to prevent epithelial migration, it can act as fibrin bandage and serve as an interpositional matrix with biological properties such as promoting neoangiogenesis, preventing necrosis and shrinkage of the surgical flap (25). It also functions as fibrin glue exhibiting space-maintaining abilities and holding the flap in a stable position (25). The $\alpha$-granules of the platelets act as a reservoir of many growth factors that play a significant role in healing and repair mechanism through cell proliferation, chemotaxis, and differentiation (26). The leukocytes entrapped within the fibrin meshwork also influence growth factor release and matrix remodeling during healing (26).

Our study reported a high success rate in the control group (80%), with significant improvement in periodontal parameters and reduction in apical radiolucency. These results are in contrast to previous studies reporting lower success (27%–37%) (3, 4) in apicomarginal defects without GTR techniques. We attribute these results to modern microsurgical techniques and biomaterials and the type of defects recruited in our study. High success rate (>60%) in apicomarginal defects without using barrier techniques has previously been reported with endodontic microsurgery (27). Also, the present study included lesions of endodontic origin with minimal or no bone loss in the proximal area. Defects involving a proximal root surface result in less favorable results to modern regenerative therapies (24). The present study evaluates the clinical usefulness of PRF in the treatment of apicomarginal defects.

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TABLE 2. PD, CAL, GMP, and SPL at Baseline and 12 Months after Surgery by Experimental Group

<table>
<thead>
<tr>
<th>Groups</th>
<th>Clinical parameters</th>
<th>Baseline, mean ± SD</th>
<th>12 Months, mean ± SD</th>
<th>$P$ value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>PD</td>
<td>9.07 ± 0.90</td>
<td>1.07 ± 0.26</td>
<td>.001*</td>
</tr>
<tr>
<td></td>
<td>CAL</td>
<td>9.67 ± 0.90</td>
<td>2.67 ± 0.98</td>
<td>.001*</td>
</tr>
<tr>
<td></td>
<td>GMP</td>
<td>−0.60 ± 0.74</td>
<td>−1.60 ± 0.91</td>
<td>.004*</td>
</tr>
<tr>
<td></td>
<td>SPL</td>
<td>105.67 ± 87.35</td>
<td>7.67 ± 9.74</td>
<td>.001*</td>
</tr>
<tr>
<td>Control</td>
<td>PD</td>
<td>8.67 ± 0.98</td>
<td>1.4 ± 0.63</td>
<td>.001*</td>
</tr>
<tr>
<td></td>
<td>CAL</td>
<td>8.80 ± 1.52</td>
<td>2.2 ± 0.68</td>
<td>.001*</td>
</tr>
<tr>
<td></td>
<td>GMP</td>
<td>−0.07 ± 0.80</td>
<td>−0.80 ± 0.68</td>
<td>.012*</td>
</tr>
<tr>
<td></td>
<td>SPL</td>
<td>111.73 ± 70.91</td>
<td>6.33 ± 8.16</td>
<td>.001*</td>
</tr>
</tbody>
</table>

SD, standard deviation.

$P < .05$ (significant).
because the periodontium is usually able to maintain its proper health in endodontic infections (29). Once the infection is removed, connective tissue attachment and/or alveolar bone may form with recruitment of stem/progenitor cells from adjacent healthy periodontal ligament, endosteum, or bone marrow (29). Furthermore, persistent root canal infection, regardless of the size of the lesion, is the prime cause of apical periodontitis after endodontic therapy (29). Therefore, a scrupulous search of the underlying pathology and its subsequent elimination aided by a microsurgical approach were taken up. Last, blood clot formed in the surgical area may have played a role because it contains growth factors/cytokines and is a good space filler or extracellular matrix (29).

In the present study PRF did not improve healing in apicomarginal defects. A possible explanation for this may lie in the fact that although it promotes healing, stimulation of particular cell types requires presentation of biomolecules in a specific stereo-spatial manner. Simultaneous release of multitude of biomolecules from PRF may not necessarily be able to provide a suitable environment for osteogenesis. It is important to point out that although the stimulatory effect of platelet concentrates is well-documented in soft tissue healing, their direct osteoinductive effect has not yet been warranted (10). Also, low fibrin content in PRF reduces its resistance to physical forces as compared with GTR membranes, thus compromising its use in stressful environments such as oral cavity (10). Furthermore, the PRF membrane is a thin scaffold and may quickly resorb in a well-vascularized environment of the oral tissues (25). Thus, to achieve the long-term stability of the stimulated tissue, the fibrin-based cica-tricial matrix must be thick and strong (25). PRF as a regenerative material presents with a few limitations such as inhomogeneity of membrane and instability of the preparation because its preparation depends on several technical factors such as the radius of centrifuge (force varies with different radii of different machines) (30) and method of compression (31). The leukocytes and platelet aggregates tend to localize within one end (toward red blood cells) of the membrane (32), particularly more so when single membrane is used. Although preparation of PRF membrane was done under aseptic conditions, avoiding any possible source of infection, chances of possible microbial or chemical contamination may still exist while handling an autologous product.

The PRF group showed significantly more PD reduction than the control group. It would not be prudent to stress on this isolated significant finding because it has been suggested that it is not necessarily related to bone loss (33). The inflammatory pseudo-pockets result in coronal displacement of gingival margin, and resolution of edema after therapy may lead to overestimation of the results. Hence, an obvious gain/loss in periodontal attachment may occur without a concomitant decrease/increase of probing PD (33).

The strengths of our study include stringent inclusion and exclusion criteria, zero dropout rate, standardization of radiographic evaluation, and use of endodontic microsurgery. This was a prospective, randomized controlled trial with the sample size calculated a priori. However, the study has certain limitations. Although 1-year follow-up may be seen as adequate to assess periapical healing (34), maintaining an extended recall may better evaluate those in the incomplete/uncertain category (7 in PRF group, 7 in the control group) to assess possible healing (35). Further healing is influenced by various factors such as variability in shape and size of the bony lesion (6), size of periapical radiolucency, etiology of defects, and duration of existing lesion, which may have confounded the results. Multiple coincident factors involved in healing also stress the need for a larger sample size. Further research with a larger sample size and long-term follow up is needed to detect the differences with greater statistical power and precision between the clinical groups. Lack of histologic evidence is another limitation of the current study because clinical and radiographic healing does not indicate true regeneration of the periodontium. Last, a three-dimensional imaging technique such as cone-beam computed tomography could have been a better diagnostic tool to assess the true nature and spatial relationship of the regenerated tissues.

Hence, the results of the present study are to be extrapolated with caution. Although our study observed high success rates of apical surgery in apicomarginal defects, even without using any barrier membrane, most of the defects recorded in our study were of primary endodontic origin, with no or minimal periodontal involvement. A further study is required to compare healing of apicomarginal defects with and without secondary periodontal involvement, represented by moderate to severe proximal bone loss.

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**TABLE 3.** Intergroup Comparison for Changes in PD, CAL, and GMP from Baseline to 12 Months and Percentage Reduction of SPL at Different Time Intervals between PRF Group and Control Group

<table>
<thead>
<tr>
<th>Clinical parameters</th>
<th>PRF, mean ± SD</th>
<th>Control group, mean ± SD</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>ΔPD</td>
<td>8 ± 0.92</td>
<td>7.27 ± 0.96</td>
<td>.046</td>
</tr>
<tr>
<td>ΔCAL</td>
<td>7 ± 0.92</td>
<td>6.6 ± 1.18</td>
<td>.265</td>
</tr>
<tr>
<td>ΔGMP</td>
<td>–1 ± 0.84</td>
<td>–0.73 ± 0.88</td>
<td>.506</td>
</tr>
<tr>
<td>3-month interval SPL</td>
<td>41.72 ± 16.83</td>
<td>41.31 ± 20.74</td>
<td>.885</td>
</tr>
<tr>
<td>6-month interval SPL</td>
<td>66.77 ± 17.83</td>
<td>62.15 ± 23.44</td>
<td>.520</td>
</tr>
<tr>
<td>9-month interval SPL</td>
<td>78.52 ± 14.95</td>
<td>79.06 ± 19.12</td>
<td>.442</td>
</tr>
<tr>
<td>12-month interval SPL</td>
<td>93.41 ± 7.00</td>
<td>94.57 ± 5.87</td>
<td>.781</td>
</tr>
</tbody>
</table>

SD, standard deviation.

*p < .05 (significant).

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**TABLE 4.** Results of Combined Clinical-Radiographic Healing 12 Months after Surgery by Experimental Group

<table>
<thead>
<tr>
<th>Radiographic healing</th>
<th>Complete (%)</th>
<th>Incomplete (%)</th>
<th>Uncertain (%)</th>
<th>Unsatisfactory (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PRF group (n = 15)</td>
<td>8 (53.33)</td>
<td>5 (33.33)</td>
<td>2 (13.33)</td>
<td>0</td>
</tr>
<tr>
<td>Control (n = 11)</td>
<td>8 (53.33)</td>
<td>4 (26.67)</td>
<td>3 (20)</td>
<td>0</td>
</tr>
</tbody>
</table>

P = .624 (non-significant).
Conclusion
Within the limits of this study, it can be concluded that a high success rate may be attained in apicorrhinal defects with endodontic microsurgery, and addition of PRF may not necessarily improve the outcome.

Acknowledgments

The authors deny any conflicts of interest related to this study.

References