Application of Platelet-Rich Fibrin and Freeze-Dried bone Allograft Following Apicoectomy: A Comparative Assessment of Radiographic Healing

Abstract
Background: Apicoectomy conceptualizes surgically maintaining a tooth with an endodontic lesion that cannot be resolved by conventional endodontic (re-) treatment. To achieve this, continuous improvement in surgical techniques, materials and instruments is being done to enhance the outcome of periapical endodontic surgeries. The purpose of this study was to compare, radiographically, the healing kinetics of platelet-rich fibrin (PRF) and mineralized freeze-dried bone allograft (FDBA) in patients undergoing apicoectomy. Materials and Methods: Nineteen patients (aged 18–40 years) were included in the study and randomly assigned to groups A or B, where they received PRF or FDBA, respectively. Following apicoectomy, PRF gel and FDBA graft were prepared and placed in the osseous defect followed by placement of PRF membrane for graft stabilization and flap closure. Radiographic follow-up was done at the 1st, 3rd, 6th and 12th months for evaluation of healing using Molven’s criteria. Statistical analysis was done with Pearson’s and McNemar’s Chi-square tests. Results: A highly significant difference (P = 0.002) in radiographic healing was observed at 6 months. Complete healing was observed in 50% of cases in Group A whereas in Group B, none of the cases presented with complete radiographic healing. However, at the end of 12 months, complete radiographic healing was observed in both groups. Conclusion: Our data suggest that PRF accelerates bone healing as compared to FDBA and is both time and cost-efficient.

Keywords: Apicoectomy, bone graft, FDBA, PRF, periapical surgery

Introduction
Curious degeneration, trauma or iatrogenic errors typically result in bacterial invasion and root canal colonization ultimately causing pulp necrosis and periapical pathology. The host’s immune response manifests this as an ‘osseous defect’, visible on the radiograph as a radiolucent region surrounding the root apex.[1] A surgical intervention (apicoectomy) is often needed to eliminate the source of periapical irritation and stimulate superior healing.[2] An apicoectomy involves resection, preparation and retrograde filling of the root end. This is preceded by complete debridement of the periapical lesion to remove any extra-radicular infection, foreign body material or cystic tissue.[3]

Over time, the reconstitution of these lost periapical tissues has been made possible using various regenerative therapies that utilized growth factors like platelet-rich fibrin (PRF), bone grafts, barrier membranes (PRF membranes, amniotic membranes), etc. It is interesting to note that PRF possesses osteoinductive properties whereas freeze-dried bone allograft (FDBA) brings about healing through osteoconduction. The primary aim of these regenerative therapies is to restore tissue architecture and function.[4]

Dohan et al.[5] described PRF as an autologous fibrin scaffold that concentrates platelets, growth factors and leukocyte cytokines of the blood harvest.[5,6] It is a biomodulator to promote both hard and soft tissue healing without significant post-surgical inflammation[7] through angiogenesis, wound healing and osteogenesis.[8]

On the other hand, FDBA stimulates healing by first undergoing resorption by the host cells. The remaining matrix serves as a scaffold for the incoming cells that can deposit new bone. Implantation of FDBA in the osseous defect may recruit the undifferentiated progenitor/stem cells to undergo osteogenesis.

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FDBA may also stimulate the pre-committed osteoblasts to form new bone.\(^9\) In light of the above-mentioned facts, the purpose of this study was to compare, radiographically, the healing kinetics of PRF and mineralized FDBA in patients undergoing periapical endodontic surgery.

**Materials and Methods**

The present study was carried out in the Department of Conservative Dentistry and Endodontics after obtaining clearance from the Institutional Human Ethics Committee (SDC/IHEC/2017/MDS-P/05) (Clinical Trial Registry Number-CT04377698). Patients aged 18–40 years with failed root canal treatment (RCT), persistent fistula or persisting symptoms after orthograde re-treatment as determined clinically and by intraoral periapical radiographs were included in the study. Whereas, patients with severe systemic disorders such as uncontrolled diabetes, immunologic diseases, malignancy, thrombocytopenia or insufficient compliance were excluded.

A power analysis was performed for sample size calculation, which mandated a minimum of nine patients in each group. However, to compensate for unforeseen dropouts, 20 patients were included in the study (10 in each group). Out of these, there was one dropout in the bone graft group who failed to return for his follow-up visits and was thus eliminated during the tabulation of results. The final recorded sample size was 19.

A thorough medical and dental history was taken and a diagnosis was made based on clinical signs and symptoms, and intraoral and radiographic findings. After obtaining informed consent, the conventional RCT was performed and the patients were alternatively assigned to the PRF group (Group-A) and the mineralized FDBA group (Group-B).

After successful administration of local anaesthesia (lignocaine hydrochloride 2%, 1:80,000), a full-thickness mucoperiosteal flap was raised by sulcular incision and two relieving vertical incisions. Curettage of the periapical tissue defect site was done using a curette, followed by a 2–3 mm root-end resection with a low-speed straight handpiece and straight fissure carbide bur. Retrograde cavity preparation was done with a contra-angled low-speed handpiece and inverted cone bur. Retrograde filling with mineral trioxide aggregate (MTA) was done using the MTA carrier and condensed to achieve a hermetic seal. The above-mentioned procedures were performed under \(2.5 \times\) magnification using Loupes (Carl Zeiss).

Venous blood (10 ml) was drawn from the patients by using an 18-gauge needle. Blood was collected in glass tubes without anticoagulant and immediately centrifuged at 3,000 rpm for 10 min, which resulted in a fibrin clot containing the platelets located in the middle of the tube, between a layer of red blood cells (RBCs) at the bottom and platelet-poor plasma (PPP) at the top. The middle layer of PRF gel was retained and placed in the osseous defect in Group A patients.

In Group-B patients, mineralized FDBA granules (Tissue Bank, Tata Memorial Hospital) were hydrated with distilled water in a Dappen dish. The graft material was then carefully placed in the defect site using a bone graft carrier and densely packed using a bone compactor.

Following this, for both groups, resistant autologous PRF membranes were prepared by extracting out the fluids trapped in the fibrin matrix using uniform compression provided by the force of the membrane tray inside the PRF box. It was then placed over the graft site followed by flap repositioning with Ethicon 3-0 Mersilk sutures to ensure complete soft tissue coverage of the graft site.

Instructions for post-operative care and oral hygiene were given. Sutures were removed 7 days following the procedure after visual evaluation of the soft tissue healing. The patients were recalled at the 1st, 3rd, 6th and 12th months for follow-up and radiographic assessment of the periapical healing [Figures 1a-e and 2a-e].

**Method of examination**

Two endodontists, extraneous to involved patients and study design, scored each follow-up intraoral periapical radiograph (IOPAR) individually and separately based on the appropriate category of the classification introduced by Molven et al.\(^{10}\) [Table 1].

The statistical analysis was done using SPSS (Statistical Package for Social Sciences) version 21.0 Statistical Analysis Software. The results were analysed using descriptive statistics and comparisons were made between both groups. Categorical data were summarized in proportions and percentages (%) while quantitative data are presented as mean ± standard deviation (SD).

**Results**

The inter-observer agreement was 100% on the evaluation of radiographs at baseline and 12 months. In the follow-up intervals, the maximum agreement was observed at 6 months follow-up (D = 0.711), followed by 3 months (D = 0.209) and 1 month (D = 0.208) follow-up. Pearson’s Chi-square test and McNemar’s Chi-square test were used for statistical analysis and it was found that according to both examiners 1 and 2, at 0 months, the PRF group and FDBA group both had scores of 0 for bone healing for all the cases. At 6 months, the PRF group and FDBA group had a significant difference in the amount of bone healing achieved (\(P = 0.002\)). PRF groups showed better results than the FDBA group. However, at 12 months, all the cases of both groups displayed complete healing [Table 2].
Discussion

The primary goal of apical surgery is to provide an ideal microenvironment for periradicular tissue healing. The surgery may involve the removal of the pathology or the inaccessible portions of the root canal system. Bone regeneration following periapical surgery is important to ensure long-term success. The desired outcome is tissue regeneration, which is the restoration or reconstitution of the lost periapical tissues. Regenerative therapies that provide a three-dimensional scaffold for cellular invasion, and contain bioactive molecules to stimulate tissue healing are increasingly being used in apical surgeries.

To the best of our knowledge, no study to date has compared the radiographic healing of bone using PRF and FDBA in teeth undergoing periapical surgery. This study is deemed to be the first of its kind and requires further extensive research to validate the same.

Table 1: Molven’s scoring criteria for radiographic healing

<table>
<thead>
<tr>
<th>Score</th>
<th>Category</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>Unsatisfactory healing</td>
</tr>
<tr>
<td>1</td>
<td>Uncertain healing</td>
</tr>
<tr>
<td>2</td>
<td>Incomplete healing</td>
</tr>
<tr>
<td>3</td>
<td>Complete healing</td>
</tr>
</tbody>
</table>

Table 2: Inter-group comparison of bone healing according to both examiners 1 and 2 combined

<table>
<thead>
<tr>
<th>Follow-up Interval</th>
<th>Score</th>
<th>PRF Group</th>
<th>Bone Graft Group</th>
<th>( \chi^2 )</th>
<th>( P )</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 month</td>
<td>0</td>
<td>100.0%</td>
<td>100.0%</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>1 month</td>
<td>1</td>
<td>70.0%</td>
<td>100.0%</td>
<td>6.41</td>
<td>0.011</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>30.0%</td>
<td>0.0%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3 months</td>
<td>1</td>
<td>35.0%</td>
<td>72.2%</td>
<td>5.27</td>
<td>0.022</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>65.0%</td>
<td>27.8%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6 months</td>
<td>1</td>
<td>0.0%</td>
<td>5.6%</td>
<td>12.75</td>
<td>0.002</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>50.0%</td>
<td>94.4%</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>50.0%</td>
<td>0.0%</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

\( \chi^2 \) = Chi-square value; \( P \) is the level of significance (>0.05 Not significant, <0.05 Significant, <0.01 Highly significant, <0.001 Very highly significant).

It has been reported that PRF can support bone healing by stimulating the extracellular-signal-regulated kinase (ERK) pathway. PRF can further support bone healing by stimulating the secretion of osteoprotegerin (OPG) in osteoblasts and suppressing the activity of osteoclasts. By upregulating both OPG and alkaline phosphatase expression, PRF can also promote osteogenic differentiation of human dental pulp cells and periodontal ligament cells. Besides being osteoinductive and osteoconductive, PRF is also highly angiogenic and is expected to significantly enhance the revascularization of the graft. The angiogenic properties are largely attributed to the three-dimensional structure of the fibrin matrix and the entrapped growth
factors such as platelet-derived growth factor and transforming growth factor.\(^{[10]}\)

PRF as a barrier membrane stabilizes the graft and prevents oral epithelium and gingival connective tissue from growing into the defect and thus facilitates natural tissue regeneration and healing. This preparation could reduce the treatment period and may assist in the decision-making of the future prognosis of periapical surgeries. Moreover, as PRF is an autologous biomaterial, it is a cost-effective alternative to other graft materials and can be harvested on demand also reducing the risk of disease transmission.

In correlation to our study (for the FDBA group), Saad and Abdellatief (1991)\(^{[9]}\) reported that osseous defects healed between 6 and 9 months, following FDBA application. During the post-operative period of 6–9 months, the grafts appeared to have completely integrated with the host tissue and could no longer be identified as an implanted material. Healing using bone allografts can be attributed to the fact that they contain bone morphogenic proteins (BMPs), which help stimulate osteogenesis. Several osteoinductive BMPs that encourage new-bone formation have been identified in bone allografts. Their activity leads to a series of developmental processes that result in the differentiation of mesenchymal cells into osteoblasts and eventually bone.

The differences observed in this study, in osseous healing, may be ascribed to the different mechanisms through which PRF and FDBA support tissue regeneration and repair. PRF and FDBA also have remarkably different material properties and resorption/remodelling rates. We speculate that the difference in healing rate could be due to faster resorption and remodelling of the platelet gel, which releases the encapsulated growth factors into the wound bed. PRF is also likely to be a more potent stimulator of angiogenesis - a critical step in the bone healing process. In contrast, FDBA takes longer to break down and remodel, which might delay the deposition of new bone. Furthermore, FDBA relies on the host for the stimulation of angiogenesis. While autogenous bone grafts are the gold standard for the reconstruction of osseous defects, the above-mentioned factors and the limited availability of autografts from intraoral sites, donor site morbidity and possible hospitalization are associated disadvantages with their use.

However, the limitations of this study were the lack of control subjects, no histologic evaluation and relatively inconsistent methods for estimating osseous regeneration. Finally, we emphasize that our results are preliminary. A long-term controlled clinical study with histological analysis and advanced radiographic techniques is necessary to confirm these findings. Future work should also focus on uncovering the timeline of degradation of these scaffolds, as well as the exact mechanism through which PRF and FDBA support bone healing.

Conclusions

Overall, this study suggests that a combination of PRF gel and membrane accelerates tissue healing, and is worthy of extensive clinical investigation in future studies. The main conclusions of this study are as follows:

- The addition of PRF and mineralized FDBA in periapical osseous defects enhances the regenerative capacity of bone.
- PRF and FDBA both are clinically advantageous as assessed by the radiographic method. In a 12-month follow-up evaluation, both PRF and FDBA presented with desirable outcomes and supported bone healing. However, in terms of healing kinetics, PRF was better than FDBA during the 6-month radiographic evaluation of bone healing in our study.
- PRF proved to be a more potent and cost-effective graft material.
- PRF membrane can synergistically enhance healing when used with adjuvants such as PRF and FDBA.

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Nil.

Conflicts of interest

There are no conflicts of interest.

References