Comparative Evaluation of Platelet-rich Fibrin and Concentrated Growth Factor as Scaffolds in Regenerative Endodontic Procedure: A Randomized Controlled Clinical Trial

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Abstract

Aim: This randomized controlled trial evaluated the efficacy of platelet-rich fibrin (PRF) and concentrated growth factor (CGF) as scaffolds in the regenerative endodontic procedure (REP) using clinical and radiographic parameters along with cone-beam computed tomographic (CBCT) analysis.

Materials and methods: The apexogenesis procedure was performed in 16 teeth. They were randomly divided into two groups of eight teeth each: group I and group II. In group I PRF was used as the scaffold and in group II CGF was used as the scaffold. They were evaluated for pain, pulpal vitality, tenderness on percussion, and mobility, and also evaluated using digital radiographs at 3, 6, 12, and 18 months interval. The response of the teeth was graded using Chen and Chen criteria. Increase in root length, reduction in the apical foramen dimension, and reduction in periapical lesion volume were evaluated using CBCT scans taken preoperatively and at 18 months.

Results: At the end of 3 months, 50% of teeth without periapical pathology were found to be vital in both groups. At the end of 18 months, 60% of the teeth in both groups showed increase in root length, all teeth showed closure of apical foramen, and reduction in the volume of periapical lesion. However, there was no statistically significant difference between the groups (p < 0.05).

Conclusion: The clinical and radiographic features reported in this study revealed that both PRF and CGF act as effective scaffolds in REP for regeneration of pulp-dentin complex with promising results.

Clinical significance: Apexogenesis by revascularization has not been used regularly for the treatment of nonvital teeth with open apex because the results are not reliable. Since platelet concentrates like PRF and CGF are rich in growth factors; when apexogenesis is performed by REP using these platelet concentrates, desirable results can be achieved in a short duration of time. They also accelerate the healing of periapical lesions present in such cases. With the increased success rate of apexogenesis with REP, many clinicians would prefer to use REP as a treatment option for teeth with open apex.

Keywords: Apexogenesis, Concentrated growth factor, Cone-beam computed tomography, Platelet-rich fibrin, Scaffolds.

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Introduction

The incidence of dental injuries is highest in the age group of 10–12 years when the root development of many teeth is not yet complete.1,2 When the pulp becomes necrotic at this stage due to trauma, root development gets arrested. These teeth have an open apex with no apical constriction. The root canal treatment of such teeth is challenging due to the lack of an apical barrier for obturation. The treatment options for such teeth include either an apexogenesis or an apexification.

In the case of apexification, cements like mineral trioxide aggregate (MTA), biodentine are used to create an apical barrier, and the remaining root canal space is obturated using gutta percha either by thermomechanical compaction or custom cone technique. But in such a case there is no further increase in root length or width making the root more prone to fracture. When apexogenesis is performed in such a case, there is continued root lengthening with the reinforcement of root canal walls by the deposition of new hard tissue.3 This in turn increases the resistance of the tooth to fracture. In open apex cases where the root length is less with thin root canal walls apexogenesis is a better treatment option since it leads to further root development.

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Apexogenesis is generally done by revascularization procedure by inducing bleeding into the root canal space using a small K file. Inducing bleeding triggers higher concentration of mesenchymal stem cells (MSCs) from the apical papilla into the root canal space. The blood clot formed by the revascularization procedure acts as a scaffold for the MSCs from apical papilla to differentiate into odontoblasts and form the root dentin.

In recent times, regenerative endodontic procedure (REP) performed using autologous platelet concentrates like platelet-rich plasma (PRP) and platelet-rich fibrin (PRF) has been used for apexogenesis of immature teeth with predictable outcomes. This REP is also referred by various terms like revascularization or revitalization. However, using CBCT, the generation of new vascular supply could not be confirmed, and the remission of pulpal vitality is not seen in all cases. So REP would be a more precise term for this procedure.

These autologous platelet concentrates are prepared from the patient’s own blood and contain higher concentration of platelets and growth factors. Therefore, when compared to blood clots, these platelet concentrates act as better scaffolds for MSCs for the regenerative process, thereby resulting in successful root development.

Concentrated growth factor (CGF) is an advanced type of second-generation platelet concentrate introduced in 2006 by Sacco. Concentrated growth factor has a denser fibrin matrix with more growth factors compared to PRF. Only a few in vitro studies that assess the effect of CGF on dental pulp cells have been performed. To the best of our knowledge, no animal or in vivo studies that substantiate the effect of CGF in apexogenesis have been performed.

The purpose of the study is to evaluate the efficiency of REP performed using PRF and CGF as scaffolds by assessing the increase in root length, closure of apical foramen, and reduction in the volume of periapical lesion using routine clinical, radiographic, and CBCT evaluation.

### Materials and Methods

The protocol was approved by the Institutional Ethical Committee of Tamil Nadu Government Dental College and Hospital (IRB no.4/IRB/2018). It followed the CONSORT guidelines (Flowchart 1). The study was conducted from March 2019 to April 2021. A total of 16 subjects (PRF = 8, CGF = 8) between the age-group of 15 and 35 years with necrotic immature teeth exhibiting radiographic evidence of open apex, such that the width of the apical foramen was greater than 1 mm [when measured radiographically using intraoral periapical radiograph (IOPA)] were chosen for the study. Teeth with open apex with pathological conditions such as chronic irreversible pulpitis, pulpal necrosis, pulpal necrosis with asymptomatic apical periodontitis, and pulpal necrosis with chronic periapical abscess were chosen for the study. Teeth with periapical lesion greater than 10 mm in diameter when measured radiographically were excluded from the study. This is because a highly infected environment would hamper the regeneration of dentinoid and cementoid material in REP.

Individuals in the age-group of 15–35 years were chosen for the study because the regenerative capacity of young individuals is considered high. Only patients with good oral hygiene and periodontally healthy teeth were included in the study. Patients with known systemic diseases, bleeding disorders, and pregnant women were excluded from the study. Patients who were allergic to ciprofloxacin, metronidazole, or minocycline were also excluded from the study. Grossly decayed or fractured teeth that were not conducive to rubber dam isolation were excluded from the study. Teeth with periodontal problems were also excluded. The subjects were randomly divided into two groups. Informed consent was obtained from these individuals.

### Study Design

For all the subjects chosen for the study, preoperative clinical, radiographic, and CBCT evaluations were done. Vitality of the pulp

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**Flowchart 1: CONSORT flow diagram**
was assessed using the electronic pulp tester (EPT) by placing it at the middle third of the crown. There was no response noted and the teeth were found to be nonvital. After administering local anesthesia, 2% lignocaine with 1:200,000 adrenaline and rubber dam isolation, the teeth were cleaned with a rubber cup and prophylactic paste at low speed. An access cavity was prepared using sterile diamond points (round bur no. 2) (Figs 1A and 2A). Working length was determined. The canal was minimally instrumented and copiously irrigated with 20 mL of 1.5% sodium hypochlorite solution for 5 minutes, 20 mL of ethylenediaminetetraacetic (EDTA) solution for 5 minutes, 5 mL of 2% chlorhexidine solution, and dried with sterile paper points. Triple antibiotic paste (TAP) was obtained by mixing equal amounts of active pharmaceutical ingredient formulation of metronidazole, ciprofloxacin, and minocycline (GAPL, Tuem, India) with distilled water to a final concentration of 0.1 mg/mL. It was placed within the root canal using a K-file and then covered with cotton pellets. The root canals were then sealed with cavity and the patient was recalled after 2 weeks.

On the second appointment, after confirming the absence of any signs or symptoms of disease, TAP was removed. The root canal was irrigated with 20 mL of 1.5% sodium hypochlorite solution for 5 minutes, 20 mL of 17% EDTA for 5 minutes followed by 5 mL of 2% chlorhexidine solution.

**PRF Preparation**
A sample of 10 mL of the patient’s venous blood was drawn and transferred to test tubes without anticoagulants. The tubes were centrifuged at 3000 rpm for 10 minutes in a centrifuge machine. The centrifugal product obtained was then removed with the help of a tweezers. It was separated from the RBC layer using sterile scissors. The obtained PRF was sectioned into pieces and packed into the root canal such that it is 3–4 mm apical to the cementoenamel junction (CEJ).

**CGF Preparation**
A total of 10 mL of venous blood was collected and transferred to sterile vacuette tubes without anticoagulant solutions. The tubes were centrifuged in a medifuge machine using a one-step centrifugation protocol at variable rpm, which consists of 30 seconds of acceleration, 2 minutes at 2700 rpm, 4 minutes at 2400 rpm, 4 minutes at 2700 rpm, 3 minutes at 3000 rpm, and 36 seconds of deceleration and stop. After centrifugation, four layers were obtained: first layer at the top is serum, second layer is the fibrin buffy coat, third layer is the liquid phase containing growth factors, and the fourth layer consists of red corpuscles. The centrifuge obtained was taken out of the test tube with a tweezer. The concentrated growth factor was obtained by cutting at the interface between the CGF and RBC layers using sterile scissors. A small amount of the RBCs is retained along with CGF, because these RBCs contain some amount of growth factors. It was packed similarly to PRF.

**Coronal Seal**
Biodentine (Septodont, India) was placed over PRF/CGF until the CEJ, with a thickness of 3–4 mm. The access cavity was then sealed using type II glass ionomer cement (GC Fuji type II).

The patients were recalled for a review at 3, 6, 12, and 18 months intervals. At every follow-up visit, the teeth that underwent REP were evaluated for clinical parameters like mobility, pain, tenderness to percussion, periodontal probing depth, and pulp vitality using EPT. At every visit, digital radiography was taken (Figs 1B and C, 2B and C), and the response of the teeth to REP was graded using the Chen and Chen grading. A CBCT evaluation was done preoperatively and after 18 months to quantify the increase in root length and thickness (Figs 3A and B, 4A and B), reduction in the size of apical foramen (Figs 3C and D, 4C and D), and reduction in the volume of periradicular lesion (Figs 3E and F, 4E and F). The CBCT data were analyzed using the Diagnocat software (Diagnocat Inc, San Francisco, CA, USA).

Mann–Whitney U test was employed to identify the statistical significance between the two groups with respect to root length, mesiodistal dimension of apical foramen, buccolingual dimension of apical foramen, Chen and Chen grading, and volume of the periradicular lesion (Fig. 5). The Chi-square test was used to find the association between the two groups for vitality status of the teeth. The p-value was set as 5% (Fig. 6).

**Results**
During the follow-up period, three patients from each group could not turn up for the follow-up visits due to the COVID-19 pandemic. The remaining 10 patients were evaluated, with 5 in the PRF group and 5 in the CGF group. All of the patients were of South Indian origin. The mean age of the participants in the PRF group was 25 years and that of the CGF group was 22 years.
Figs 2A to C: Apexogenesis with CGF (A) access opening done in 11; (B) immediate postoperative IOPA following REP; and (C) postoperative IOPA at 18 months.

Figs 3A to F: A CBCT evaluation of the tooth following apexogenesis with PRF (A) pre-op tooth length, (B) post-op tooth length, (C) pre-op apical foramen width, (D) post-op apical foramen width, (E) pre-op periapical lesion volume, and (F) post-op periapical lesion volume.

Figs 4A to F: A CBCT evaluation of the tooth following apexogenesis with CGF (A) pre-op tooth length, (B) post-op tooth length, (C) pre-op apical foramen width, (D) post-op apical foramen width, (E) pre-op periapical lesion volume, and (F) post-op periapical lesion volume.
The primary goal of an apexogenesis procedure is continued root development and apical end closure. This root development happens due to the MSCs from the apical papilla, which differentiate into odontoblasts in the presence of a desirable matrix. In the present study, platelet concentrates like PRF and CGF have been used as scaffolds for the REP.

Platelet-rich fibrin belongs to the second generation of platelet concentrates. It was first prepared in 2001 by Choukroun et al. Platelet-rich fibrin releases a significant amount of various cytokines, such as transforming growth factor-β (TGF-β1), platelet-derived growth factor (PDGF), and vascular endothelial growth factor (VEGF) that potentiate further root development as the fibrin matrix is resorbed. Transforming growth factor-β plays a vital role as signaling molecules for the odontoblastic differentiation during tooth development. Similarly they also act as important signaling molecules for odontoblasts differentiation in apexogenesis. The angiogenic growth factors like PDGF and VEGF in the PRF matrix stimulate new capillary formation during the apexogenesis procedure. Due to these advantages, PRF has been successfully used as a scaffold in the apexogenesis procedure.

Concentrated growth factor is an advanced type of second-generation platelet concentrate. It was first synthesized by Sacco in 2006. Concentrated growth factor is prepared using an altered centrifugation speed from 2,400 to 2,700 rpm. This results in a denser matrix with increased amount of growth factors than those observed in PRF or PRP. This is because PRF is prepared using a constant centrifugation speed unlike CGF. It has fiber structure that is more closely packed and so is relatively stiffer than PRF or PRP. It also exhibits sustained release of growth factors for up to 2 weeks, with a peak concentration on 5th day.

Rodella et al. demonstrated that growth factors like TGF-β1 and VEGF which are highly essential for stimulating cell proliferation, matrix rehabilitation, and angiogenesis were found abundantly in CGF. Immunohistochemical analysis also showed the presence of CD34+ cells in CGF, these cells have a significant role to play in maintaining the vascular supply, neovascularization, and angiogenesis. The contents of CGF are more easily quantified since the centrifuge conditions of CGF are maintained constant. Therefore when CGF is used in clinical scenarios the outcomes are also more predictable.

There have been a few in vitro studies that evaluate the effect of CGF on the stem cells of apical papilla (SCAP), but no in vivo or randomized clinical trials regarding the role of CGF in REP have been reported yet.

In our study following the apexogenesis procedure, the patients were reviewed at regular intervals for 18 months. None of the patients had any pain, tenderness to percussion, or mobility associated with the teeth during the follow-up period. In teeth without any periapical lesion, remission of pulpal vitality was noted in 50% of the cases in both groups. But there was no statistically significant relationship observed between the vitality status of the two groups ($p = 0.490$).

Increase in the root length was noted in 60% of the teeth in both groups. Reduction in the size of apical foramen and reduction in the volume of periapical lesion was noted in 100% of the teeth in both groups. However, there was no statistically significant difference between the two groups with respect to any of these parameters.

With respect to Chen and Chen grading (Table 1), 20% of the teeth in group I showed type I response, while 40% showed type II and type IV response. In group II, 40% of the teeth showed type I and type V responses, while 20% showed type II response. However, there was no statistically significant difference between the two groups with respect to Chen and Chen criteria ($p = 0.914$).
the patients in both the groups. This is similar to a case report of revitalization using PRF published by Shivashankar et al.,19 where they observed further root development and healing of the periapical lesion at 1-year follow-up. They hypothesized that usually some SCAP remain vital even in case of a large periapical lesion. Once the infection and inflammation are eliminated, under the influence of Hertwig’s epithelial root sheath, these cells differentiate into odontoblasts like cells depositing dentinoid and cementoid material.

In CGF group, faster root development was noted compared to PRF group at 3 months, but at the end of 18 months this difference was negligible. This is because CGF has a denser fibrin matrix that is richer in growth factors compared to PRF.

In a review on CGF, Li et al. have also concluded that CGF is better than its predecessors in terms of its composition and efficacy.20 A similar case report of revascularization using CGF was published by Niveditha et al.,21 where they observed an increase in the root thickness, apical foramen closure, and reduction in the size of periapical lesion at 1-year follow-up.

No statistically significant difference was observed between the two groups. An in vitro study was performed by Hong et al.22 evaluating the proliferation, migration, and mineralization by SCAP in PRF and CGF conditioned medium. They concluded that both PRF and CGF had stimulatory effect on the SCAP.

The limitations of this study are the smaller sample size due to the corona pandemic outbreak, and the short-term clinical follow-up of 1.5 years.

**CONCLUSION**

Both groups showed further root development, reduction in the size of apical foramen, and healing of the periapical lesion, however no statistically significant difference was noted between the two groups. Therefore, the REP performed using autologous platelet concentrates like PRF and CGF seems to have promising results.

Further studies on large sample size for longer follow-up periods may open newer prospects in the future, regarding the use of REP using platelet concentrates.

**REFERENCES**


**Table 1:** Chen and Chen grading of response to REP

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<thead>
<tr>
<th>Types</th>
<th>Radiographic apex</th>
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<tr>
<td>Type I</td>
<td>Increased thickening of the canals and continued root maturation</td>
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<tr>
<td>Type II</td>
<td>No significant continuation of root development with root apex becoming blunt and closed</td>
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<tr>
<td>Type III</td>
<td>Continued root development with the apical foramen remaining open</td>
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<tr>
<td>Type IV</td>
<td>Severe calcification (obliteration of canal space)</td>
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<tr>
<td>Type V</td>
<td>A hard tissue barrier formed in the canal between the coronal MTA plug and the root apex</td>
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