Regenerative Evaluation of Immature Roots using PRF and Artificial Scaffolds in Necrotic Permanent Teeth: A Clinical Study

Neelam Mittal¹, Vijay Parashar²

ABSTRACT

Aim: The aim of this study is to evaluate and compare the regenerative potential of natural scaffold [platelet-rich fibrin (PRF)] and artificial scaffolds (commercially available collagen, placentrex, and chitosan) in necrotic immature permanent teeth.

Materials and methods: Necrotic immature permanent maxillary incisors with or without radiographic evidence of periapical lesion were included. Access opening was done under rubber dam isolation. Canal disinfection was done using minimal instrumentation, copious irrigation, and double antibiotic paste as interappointment medicament for 4 weeks. After 4 weeks, asymptomatic teeth were divided into four groups on the basis of scaffolds used for the revascularization procedure: group I (PRF); group II (collagen); group III (placentrex); group IV (chitosan). The clinical and radiographic evaluations of teeth were done at 3, 6, and 12 months after the procedure and compared with baseline records.

Results: Clinically, patients were completely asymptomatic throughout the study period. Radiographically, all cases showed an improvement in terms of periapical healing, apical closure, root lengthening, and dentinal wall thickening. PRF and collagen gave better results than placentrex and chitosan in terms of periapical healing, apical closure, and dentinal wall thickening.

Conclusion: Revascularization procedure is more effective and conservative over apexification in the management of necrotic immature permanent teeth. This study has shown that PRF and collagen are better scaffolds than placentrex and chitosan for inducing apexogenesis in immature necrotic permanent teeth.

Keywords: Open apex, Revascularization, Scaffold.

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INTRODUCTION

Injury to an immature tooth leading to pulp necrosis may result in incomplete root formation. It can interfere with further root development leaving a tooth with thin root canal walls. Weak teeth having thin walls are susceptible toward fracture. Teeth with immature apex have various anatomical complexities and, hence, conventional root canal treatment techniques used in mature teeth are difficult to perform in such cases. The instrumentation and obturation in immature teeth are difficult with conventional techniques. Conventional apexification is a successful treatment option for immature teeth but it has many disadvantages, e.g., it does not cause an increase in the root length or dentinal thickness and it also alters mechanical properties of dentin, rendering it more susceptible to fracture. An alternative biological-based treatment known as the regenerative endodontic therapy has been introduced for immature teeth with the necrotic pulp. Procedures preserving the remaining dental pulp stem cells and mesenchymal stem cells of apical papilla can result in intracanal revascularization with continuous development of root.

Regenerative endodontic procedures have the potential to allow continuous root development and can, therefore, offer an alternative approach for the management of immature permanent teeth with compromised structural integrity.

Hargreaves et al. have identified three components that are essential for the success of tissue engineering. They include stem cells that are capable of hard tissue formation, signaling molecules for cellular stimulation, proliferation, and differentiation, and, finally, a 3-dimensional physical scaffold that can support cell growth and differentiation.

One of the most important components of tissue engineering is scaffolds. Scaffolds provide support for cell organization, proliferation, differentiation, and vascularization. Ideal requisites for a scaffold are that it should be porous (to allow for placement of cells and growth factors), should be biocompatible with the host tissues, should have correct shape and form to allow for the replacement of the lost tissues, and should be biodegradable, leaving no toxic by-products. Many types of biodegradable or permanent natural [blood clot, platelet rich fibrin (PRF), hyaluronic acid, chitosan, and chitin] or synthetic (polylactic acid, polyglycolic acid, tricalcium phosphate, and hydroxyapatite) scaffolds have been used to regenerate dentin or dentin–pulp complexes in combination with dental pulp cells (DPCs). Natural scaffolds have the advantage of good biocompatibility and bioactivity, whereas synthetic scaffolds have more control over the degradation rate and have better mechanical properties.

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¹²Faculty of Dental Sciences, Institute of Medical Sciences, Banaras Hindu University, Varanasi, Uttar Pradesh, India

Corresponding Author: Vijay Parashar, Faculty of Dental Sciences, Institute of Medical Sciences, Banaras Hindu University, Varanasi, Uttar Pradesh, India, Phone: +91 9936033339, e-mail: parasharvj.kgmc@gmail.com


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There are very few clinical studies that have compared the scaffolding ability of natural (i.e., PRF) and artificial scaffolds. The present study was aimed to compare natural scaffolds, i.e., PRF, and artificial scaffolds, i.e., collagen, placentrex, and chitosan for the clinical outcome of the treatment.

**Material and Method**

The clinical study was performed at the Department of Conservative Dentistry and Endodontics, Faculty of Dental Sciences, Banaras Hindu University, Varanasi, India. After taking approval from the Ethical Committee of the Institute and explaining detailed treatment protocol to the patients, revascularization procedure was performed in 16 cases of necrotic immature permanent teeth using PRF, collagen, placentrex, and chitosan as four different scaffolds. Patients with necrotic immature permanent maxillary incisors with open apex with or without radiographic evidence of periapical lesion were selected. Medically compromised patients with systemic disorders that can lead to delayed healing or can cause bleeding disorders were excluded.

Access opening was done in teeth under rubber dam isolation using #2 round diamond bur (Endo Access Bur, DENTSPLY Maillefer) and axial wall extensions were made with a safe tip fissure carbide bur (Endo-Z Bur, DENTSPLY Maillefer). Necrotic tissue from the canals was removed by minimal canal instrumentation that also prevented further weakening of the lateral dentinal walls. Copious irrigation of the canals with 2.5% of sodium hypochlorite solution (Nimai Dento India) was done. The double antibiotic paste was used as the interappointment medicament for further disinfection of the root canals. The access cavity was then sealed with the temporary sealing material Cavitemp (AMDENT, Mohali, India) for 4 weeks.

Teeth were reaccessed under rubber dam isolation after 4 weeks. Copious irrigation with 2.5% of sodium hypochlorite solution was used to remove the double antibiotic paste from the canals. Canals were then dried to perform further revascularization procedure only in the tooth which was asymptomatic with no drainage from the canal.

Sixteen cases with necrotic immature permanent maxillary incisors, selected for the study, were randomly divided into four groups per the use of scaffold for revascularization.

**Group I: PRF**

PRF was prepared by drawing 5 mL of venous blood from the patient, collected in dried glass test tube, and centrifuged at 2,700 rpm for 12 minutes. Platelet activation and fibrin polymerization were triggered immediately because of the absence of anticoagulants. After centrifugation, three layers were formed in the test tube—base layer of RBCs, top layer of acellular plasma, and a PRF clot in the middle. The clot was then gently pressed between two-gauge pieces to form a membrane. The tooth was reaccessed. PRF was introduced into the canal and carried to the apical part of the root canal using an endodontic plunger. Access cavity was then sealed with the glass ionomer cement followed by a composite resin (Fig. 1).

**Group II: Collagen**

The teeth in this group were reaccessed as mentioned for group I. Bleeding was induced in the canal by periapical instrumentation with #30k file to form a blood clot in the root canal below the cementoenamel junction. Sterile collagen granules were then inserted into the root canal and pushed with the help of an endodontic plunger. Access cavity was sealed with the glass ionomer cement followed by a composite resin (Fig. 2).

**Group III: Placentrex**

The teeth in this group were reaccessed as mentioned for group I. Blood clot was induced in the root canal as done in group II. Sterile collagen granules mixed with placentrex were inserted into the canal and pushed inside with an endodontic plunger. Access cavity was sealed with the glass ionomer cement followed by a composite resin (Fig. 3).

**Group IV: Chitosan**

The teeth in this group were reaccessed as mentioned for group I. Blood clot was induced in the root canal as done in group II. Sterile chitosan granules were inserted into the canal and pushed inside with an endodontic plunger. Access cavity was sealed with the glass ionomer cement followed by a composite resin (Fig. 4).

An intraoral periapical radiograph was taken for the base line record. The radiographic evaluations of teeth were done at 3, 6, and 12 months after the procedure and compared with the base line records for periapical healing, apical closure, dentinal wall thickening, and root lengthening (Table 1). The cases were clinically and radiographically evaluated by two independent observers who were blinded from the groups.

The scoring criteria used for radiographic evaluation were as follows: no improvement from baseline was denoted by 0, fair improvement from baseline by 1, good improvement from baseline by 2, and excellent improvement from baseline by 3. The data were analyzed by the one-way ANOVA test of significance and Z test for proportions and $p < 0.05$ was considered statistically significant.

Figs 1A to D: Radiographs of teeth showing (A) Preoperative status; (B) Status after 3 months, (C) Status after 6 months; and (D) After 12 months of using PRF as a scaffold.
Results

On clinical and radiographic evaluation all the 16 cases showed improvement compared to the baseline levels after 12 months follow up. All patients remained completely asymptomatic throughout the study period showing no signs of tenderness to palpation or percussion. The cases with the preoperative presence of swelling or sinus exhibited complete resolution after treatment. Clinically, all the groups showed excellent results.

All the 16 cases on radiographic evaluation also showed improvement in terms of periapical healing, apical closure, root
lengthening, and dentinal wall thickening. The normal probability plot showed the normality in behavior of composite scores among the four groups. Therefore, one-way ANOVA test was applied to see the significance of difference between the groups.

**Periapical Healing**

While assessing periapical healing, collagen gave best results with 25% of cases showing excellent periapical healing and 75% of cases showing good healing. This was followed by group I (PRF) which showed excellent results in 25% of cases and good healing in 50% of cases. This was followed by group IV (chitosan) showing good healing in all the cases. Least periapical healing was seen in group III (placentrex) with 50% of cases showing good healing and 50% of cases showing only fair amount of periapical healing (Table 1 and Fig. 5). When the one-way ANOVA test was applied to these results, \( p \) value = 0.330 was observed which implies that there is no statistically significant difference between these groups in terms of periapical healing.

**Apical Closure**

While assessing apical closure, group I (PRF) gave best results, with 25% of cases showing excellent results and 50% of cases showing good results. It was followed by group III (placentrex) with 25% of cases showing excellent apical closure and 25% of cases showing good closure. It was followed by the collagen group (group II) which showed excellent closure in 25% of cases. The chitosan group was the least effective in apical closure with only 25% of cases showing good results (Table 1 and Fig. 6). When one-way ANOVA was applied to these results, \( p \) value = 0.634 was observed which means that there is no statistically significant difference between these groups in terms of apical closure.

**Root Lengthening**

Assessment of root lengthening showed that group III (placentrex) gave the best results with 75% of cases showing good root lengthening. This was followed by group II (collagen) and group IV (chitosan) both showing good root lengthening in 25% of cases. PRF (group I) gave the least effective results with all the cases showing only fair amount of root lengthening (Table 1 and Fig. 7). When one-way ANOVA was applied to these results, \( p \) value = 0.152 was observed which means that there is no statistically significant difference between these groups in terms of root lengthening.

<p>| Table 1: Comparative evaluation of different scaffolds for different parameters |</p>
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Dentinal Wall Thickening

While evaluating dentinal wall thickening, group I (PRF) showed best results with all the cases showing good amount of thickening. The collagen group was next with 75% of cases showing good dentinal wall thickening. This was followed by group IV (chitosan) with 50% of cases showing good results. Group III (placentrex) was the least effective with all the cases showing only fair amount of dentinal wall thickening (Table 1 and Fig. 8). When one-way ANOVA was applied to these results, p value = 0.018 was observed which means that there is statistically significant difference between these groups. The Z test for proportions was then applied to assess which groups had significant difference among them. According to the Z test for proportions, only the difference between groups I and III (p = 0.003) and between groups II and III (p = 0.017) was statistically significant. This implies that group I (PRF) and group II (collagen) were better than group III (placentrex).

Discussion

The treatment of an immature tooth with necrotic pulp is challenging to endodontist with potential complications. Standard biomechanical preparation with endodontic files is not advised due to thin walls. Treatment options like calcium hydroxide apexification (stimulate formation of hard tissue barrier) and MTA apexification (creates artificial barrier) have been used with limitations.

An ideal treatment approach of immature teeth with necrotic pulps and apical periodontitis would be a regenerative approach that induces the formation of an endogenous mineralized structure within the canal space with the aid of stem cells at the apical region.19 There are certain advantages for revascularization procedures. After control of infection, revascularization can be completed in a single visit. It is time effective and cost effective as compared to other techniques. The major advantage of this method is continued deposition of new dentin/hard tissue along the lateral dentinal walls resulting in continued root development and strengthening of the root with an increased crown root ratio.12

A proper scaffold material is a core component in the regenerative procedure as it provides site for stem cell adhesion, support cell proliferation, and differentiation. Ideal requirements of scaffold are their selective binding and localization of cells, contain growth factors, and undergo biodegradation over time.

In the present study, four different scaffolds PRF, collagen, placentrex, and chitosan were used. The results with PRF and collagen were better than placentrex and chitosan.

PRF was selected for this study because of its advantages over other platelet concentrates. The technique for obtaining PRF is quite simple involving less armamentarium and less time consuming as well.

PRF developed in France by Choukroun et al.16 belongs to the second-generation platelet concentrate. Su et al.17 suggested that because the maximum release of growth factors (PDGF-AB, TGF-β1, VEGF, and EGF) from PRF is in first 60 minutes, it should be used on the surgical sites preferably before this period has elapsed. The present study followed this protocol and prepared PRF was used within this period. Gassling et al.18 concluded that PRF has higher periosteal cell proliferation rates compared to collagen. The higher periosteal cell proliferation rates of PRF can also be attributed to the release of growth factors from platelets in PRF that has direct influence on cell proliferation. In the present study also, PRF gave the best results for most of the observed parameters. About 75% of all the cases exhibited good or excellent periapical healing and apical closure. About 100% of cases exhibited good dentinal wall thickening. However, in terms of root lengthening, PRF could not show good results as all the cases showed only fair amount of root lengthening. Huang et al.19 showed in a study that viability of DPCs is not affected by PRF.

Collagen type I is a natural component of human dental pulp. Similarity to this type I collagen was the reason why commercially available collagen was selected as scaffold. Additional advantages were its ease of availability and ease of use. A collagen scaffold allows tissue regeneration by its own natural degradation. Nevins et al.20 in their study filed a partially vital immature maxillary lateral incisor with a cross-linked collagen gel after total pulpectomy. Treatment outcome showed complete root development and hard tissue formation on radiographic evaluation. Thibodeau et al.21 in a study induced blood clots with and without collagen solution after disinfecting the canal. It was found that blood clot induction helped the formation of mineralized tissue in the canal space, but there was no improvement in tissue formation after the addition of collagen solution. This was probably because of quick resorption of collagen solution that could not provide any stable scaffold. Yamauchi et al.22 in a study of immature teeth with apical periodontitis used cross-linked collagen with an induction of bleeding. Results showed a significant increase in the formation of mineralized tissues. Improved healing of the apical periodontitis in the collagen scaffold group was attributed to the Osseo-inductive properties of the collagen. Sumita et al.23 demonstrated in vivo complete tooth morphology with root-like structures in the implants obtained from the collagen sponge scaffolds. Collagen fibers are thought to function as a “trap” for osteoinductive factors such as bone morphogenetic protein (BMP), TGF-β, IGF-I, and other cytokines and, thus, mediate osteogenic differentiation.24 In our study, collagen was used along with blood clot and was found to give results comparable to that of PRF. About 100% of cases showed good or excellent periapical healing, 25% of cases showed excellent apical closure, 25% of cases showed good root lengthening, and 75% of cases showed good dentinal wall thickening.

Placentrex is a drug containing peptides (FNP-III, CRF), nucleotides (PDRN, NADPH), and glutamate and is derived from an extract of fresh term, healthy, human placenta. This is the most available but least investigated material and contains a wide range
of biologically active substances and materials. Shankar et al. investigated the cytotoxicity of bio-dentine cement mixed with a new potential regenerative scaffold hydrogel (placentrex) to periodontal ligament (PDL) fibroblast cells. Results showed that both bio-dentine and placentrex are highly bio-compatible materials; bio-dentine coated with the placentrex gel was least cytotoxic and showed an increased proliferation of cells.

Placentrex has so far been used in the fields of obstetrics, gynecology, surgery, and orthopedics mainly for its wound healing, tissue regeneration, and antimicrobial properties. Placentrex is known to express fibronectin like activity, produces cross linkage along with fibrin to form a plug, and can, thus, serve as a scaffold in regenerative therapy. For these reasons, placentrex was taken as one of the scaffolds in our study. In our study, 50% of cases showed good periapical healing, 50% of cases showed good or excellent apical closure, 75% of cases showed good root lengthening, and 100% of cases showed only fair dentinal wall thickening.

Chitosan, chemically similar to cellulose, has been investigated for use as a scaffold for dental pulp regeneration. Tsunenori Matsunaga et al. demonstrated that chitosan monomer can be used as a pulp capping medicament. Wu et al. conducted a study to develop antibacterial polyelectrolyte complex scaffold that may be a good alternative for dentistry. A study concluded the use of antibacterial PEC in treating dental bone defects. Hatab et al. conducted an in vivo and immunohistochemical study of dentin and pulp tissue regeneration in the root canal using dental pulp stem cells combined with chitosan as a scaffold material. A study concluded the possibility of regenerating pulp like tissue after an endodontic procedure. Chitosan has been shown to be one of the most promising biomaterials for orthopedic and dental applications.

In our study, 100% of cases showed good periapical healing, 25% of cases showed good apical closure, 25% of cases showed good root lengthening, and 50% of cases showed good dentinal wall thickening.

**Conclusion**

Revascularization procedure is more effective and should be preferred over apexification in the management of necrotic immature permanent teeth. This study has shown that PRF and collagen are better scaffolds than placentrex and chitosan for revascularization procedure in immature necrotic permanent teeth. Tissue engineering has a great potential in the treatment of various complex diseases of teeth. Further studies focusing on the use of stem cells, scaffolds, growth factors, gene therapy, etc., in root canal therapy will be helpful in providing a way for the regeneration of vital tissue within the necrotic root canal spaces.

**Clinical Significance**

Vital teeth are less susceptible to caries compared to that of root canal-treated teeth. Regenerative endodontic therapy aids in regaining the tooth viability and its sensibility to improve in function. There are very few clinical studies that have compared the scaffolding ability of natural and artificial scaffolds.

**References**


