Original Article

Comparison of double antibiotic chitosan hydrogel scaffold with platelet-rich fibrin in regeneration in immature necrotic permanent teeth – Randomized controlled trial

Neelam Mittal, Harakh Chand Baranwal, Harshitaa Aggarwal, Shubham Kharat, Silviya Samad, Aiyman Ayubi

Department of Conservative Dentistry and Endodontics, Faculty of Dental Sciences, Institute of Medical Sciences, BHU, Varanasi, Uttar Pradesh, India

Abstract

Background: Fibrin, a natural hydrogel, can act as scaffold for tissue regeneration. Antibiotic-loading of hydrogels can create an infection-free environment for stem cell proliferation and maturation.

Aim: To compare regenerative endodontic potential of three groups (antibiotic-loaded hydrogel, chitosan-loaded hydrogel, and double antibiotic-loaded chitosan hydrogel) in immature necrotic permanent teeth with platelet rich fibrin (PRF).

Materials and Methods: Fifty-six immature necrotic teeth with periapical lesions were included in simple randomized parallel-controlled superiority trial. Patients aged 10–35 years were assigned into four groups based on scaffolds used: Group 1 (PRF), Group 2 (double antibiotic-loaded [metronidazole and ciprofloxacin] chitosan hydrogel), Group 3 (double antibiotic-loaded hydrogel), and Group 4 (chitosan-loaded hydrogel). One patient per group was lost to follow-up. Regenerative outcomes (change in apex size, root length, radicular dentin thickness, and periapical healing) were assessed at 12 months, through double-blinding and compared postprocedure using cone-beam computed tomography.

Statistical Analysis: Based on normality distribution, change in apex size (ANOVA test) while root length, radicular dentin thickness (Kruskal–Wallis test), and periapical healing (paired *t*-test) were used.

Results: Group 2 showed superior regenerative outcomes ($P = 0.001$) compared to groups 1, 4 after 12 months, and no significant difference with Group 3.

Conclusion: Double antibiotic-loaded chitosan produced significantly superior outcomes compared to PRF in promoting apexogenesis in immature permanent teeth with necrosed pulp.

Keywords: Chitosan; double antibiotics; fibrin hydrogel; platelet-rich fibrin; regenerative endodontic procedure

INTRODUCTION

"Regenerative endodontics" is a biological method for restoring damaged dental structures-dentin, root, and pulp–dentin complex cells.[1] Scaffolds guide tissue regrowth, offering biocompatibility and support for cell activities.[2,3] Natural scaffolds also offer specific cell interactions, example Choukroun's platelet rich fibrin (PRF).[3-5]

Hydrogels are the three-dimensional network of hydrophilic polymers.[6] Fibrin, a natural hydrogel, involved in clot formation can act as scaffolds for tissue regeneration with

Address for correspondence:
Dr. Harshitaa Aggarwal,
Floor No-3, Room No-6, Faculty of Dental Sciences, Institute of Medical Sciences, BHU, Varanasi - 221 005, Uttar Pradesh, India.
E-mail: aggarwalharshitaa@yahoo.in

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nontoxic degradation,[7] also for the formation of dental pulp-like tissue.[8]

Despite all the recent advances in regenerative endodontic procedures (REP), persistent infection remains a constant challenge leading to treatment failure.[9]

Antibiotic-loaded chitosan fibrin hydrogels can act as dual-function scaffolds, fostering infection-free environment and enhancing cellular functions. Combining chitosan with fibrin hydrogel gives it antibacterial properties.[8] In addition, this combination extends drug release cycles, such as for ciprofloxacin, by interacting with chitosan, all without harming stem cells.[8,10] These can render regenerative procedures more predictable and enable single-visit treatments. There remains a lacuna in the knowledge of the clinical efficacy of antibiotic-loaded hydrogels in REP due to absence of clinical trials.

This research aims to explore the efficacy of double antibiotic-loaded fibrin gels with and without chitosan integration to enhance the outcomes of REP. The research hypothesis is that using chitosan-fibrin gel loaded with low concentrations of antibiotics will improve clinical results in REP in comparison to PRF. While null hypothesis is that there is no significant difference in regenerative potential between the groups.

MATERIALS AND METHODS

Study design, settings, and ethical approval

A randomized parallel controlled superiority clinical trial based on PRIRATE 2020 guidelines was designed. Ethical approval was taken from the ethical committee of the institute (ID 2022/EC/3461) and research was registered with Clinical Trials Registration (CTRI/2022/11/04). There were no changes made in the trial/study design later.

Eligibility criteria

Patients aged 10–35 years (both sexes) and from different economic background, with necrotic immature permanent single-rooted teeth with <3 mm pocket depth and no mobility. Radiographs displayed open apex with or without periapical radiolucency. During the clinical examination, responses to heat and cold sensitivity tests were negative. Patients with cardiac diseases, pregnancy, lactation, diabetes, bleeding disorders, HIV, or hepatitis-B positivity were excluded from the study.

Sample size calculation

A total sample size of 52 (13/group) was determined using G*Power 3.1.9.4 software (Heinrich Heine University, Germany), for 95% study power at significance of 0.05 and effect size of 0.505. This sample size adequacy was corroborated by the data from a prior study.[11]

Randomization

Fifty-six necrotic permanent teeth with open apex that reported in the Department of Conservative Dentistry and Endodontics were chosen. Participants were randomly assigned to groups-PRF, Double Antibiotic-loaded Fibrin Hydrogel, Chitosan-loaded Fibrin Hydrogel, and Double Antibiotic-loaded Chitosan Fibrin Hydrogel using simple randomization based on the computer-generated number sequence using a software program available at http://www. random.org [Figure 1].

Preparation and characterization of the scaffolds

Fibrin hydrogel scaffolds were prepared based on the protocol in the study by Hacer Aksel *et al*.[12] by mixing fibrinogen (12.5 mg/mL; Millipore Sigma), calcium chloride (2.5 mmol/L; Millipore Sigma), and thrombin (4 U/mL; Millipore Sigma). Chitosan (Polysciences, 15,000 MW) was dissolved in 1% acetic acid (v/v) and integrated into the fibrin gel at a concentration of 0.4%. A pH of 7 was achieved by adding 32 mmol/L β-glycerophosphate disodium salt hydrate (β-GP; Millipore Sigma). For the antibiotic groups, equal parts of metronidazole and ciprofloxacin powders were utilized at a concentration of 0.40 mg/L dissolved in 0.1N acetic acid.

The viscosity of the chitosan-fibrin formulation at 37°C was studied and it was found to undergo complete gelation in approximately 7 min (±2), for a concentration of 0.4% chitosan.

The prepared scaffolds underwent nano-structural morphology examination using scanning electron microscopy [Figure 2a-c].

Ultraviolet spectrophotometry was used to study the release of antibiotics from the hydrogel scaffolds. The release patterns were examined in PBS at 37°C in a 5% CO2 environment over a period of 7 days [Figure 3a and b].

The prepared scaffolds were stocked at 4°C in sterile syringes [Figure 4] until clinical usage to avoid premature gelation.

Clinical procedure

The procedure was explained to the patients, (or to parents, if below 14 years) and written consent was obtained. Opaque and sealed envelopes were prepared with different treatment groups mentioned and the patient selected one envelope randomly.

Using basic instruments and under local anesthesia and rubber dam isolation, an access opening was made with a #2 round diamond bur, followed by axial wall modifications using a safe tip Endo-Z bur [Figure 5a]. Minimal instrumentation of the canal was performed to prevent further weakening

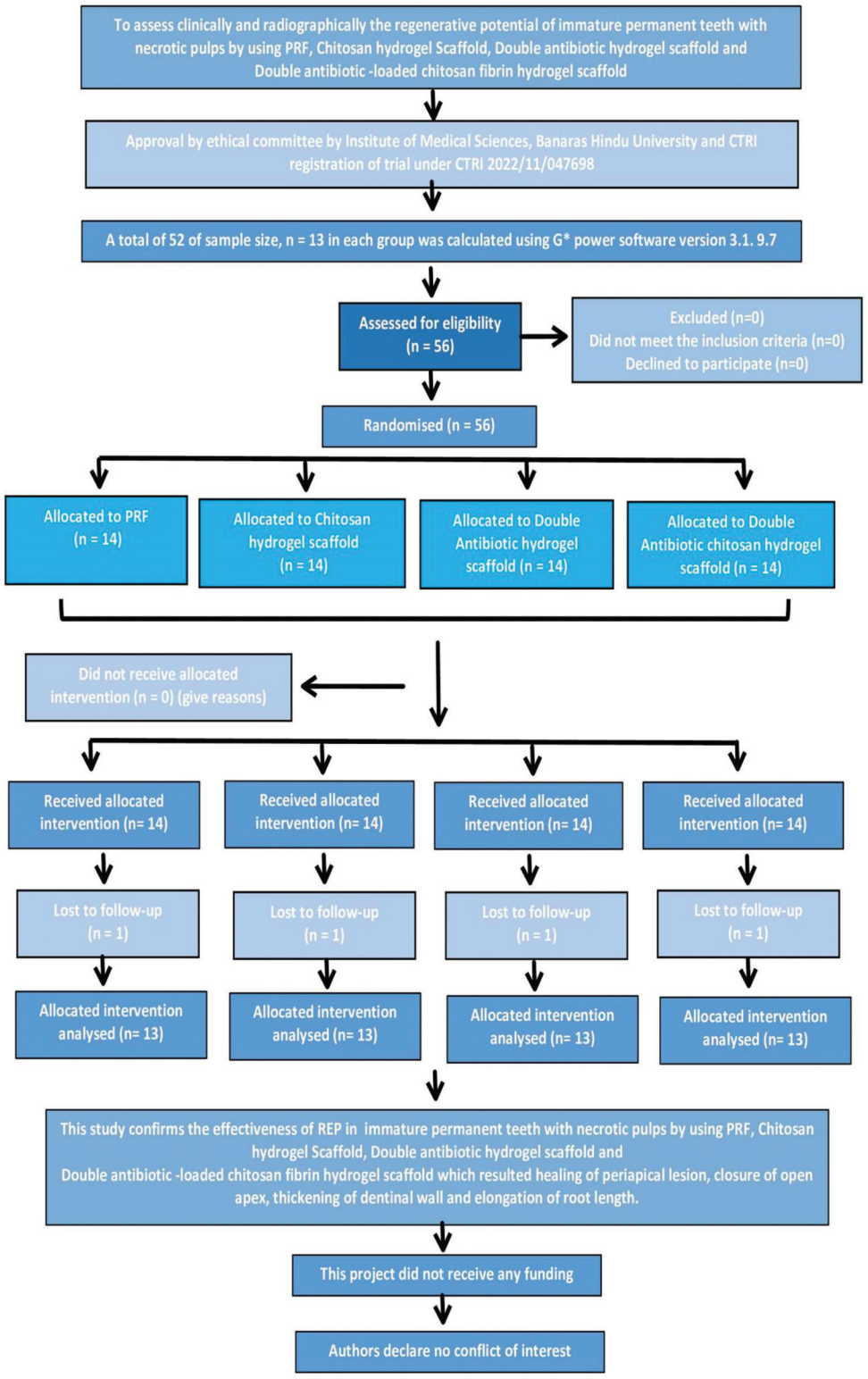


Figure 1: Preferred Reporting Items for Randomised Trials in Endodontics 2020 flowchart of participant

of the lateral dentinal walls while removing necrotic tissue. Following that, the canals were thoroughly flushed with a 20 ml solution of 1.5% sodium hypochlorite and 10 ml of sterile saline, using a 29-G side-vented needle. After

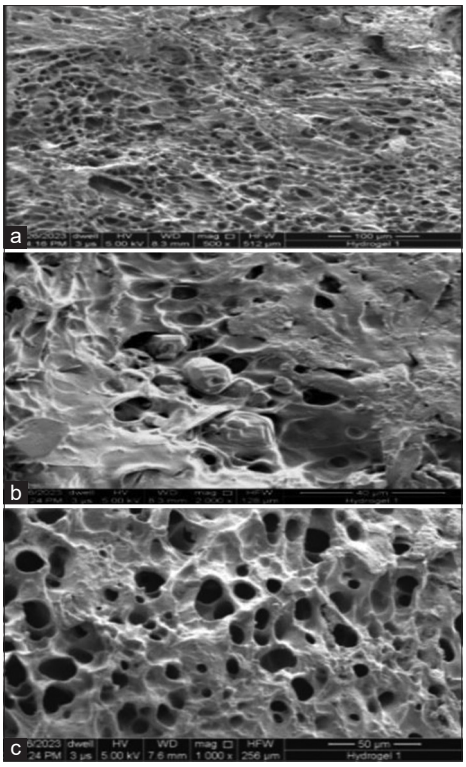


Figure 2: (a-c) Structure of double-antibiotic loaded chitosan hydrogel under scanning electron microscope at different magnifications (×500, ×1000, and × 2000, respectively)

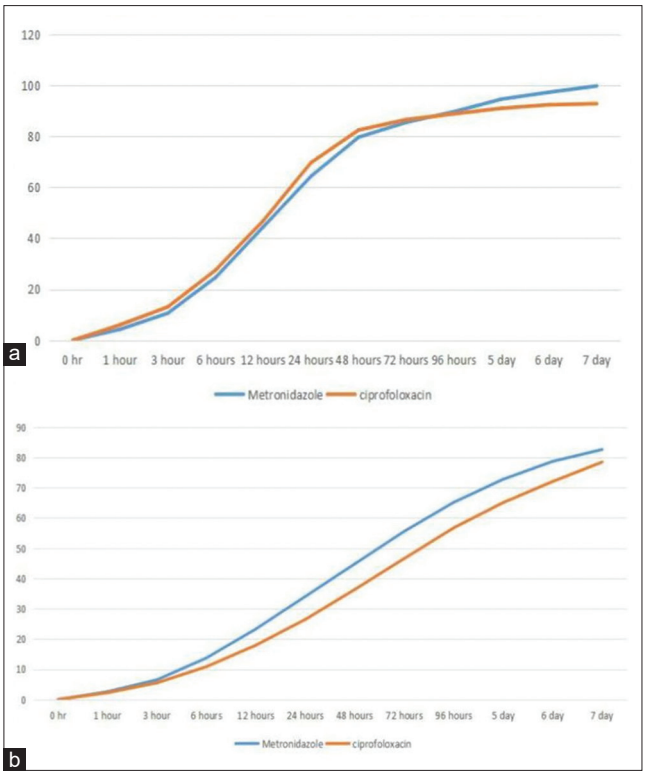


Figure 3: (a) Cumulative release (%) of antibiotic (metronidazole and ciprofloxacin) at 0.40 mg/L in fibrin gel. (b) Cumulative release (%) of antibiotic (metronidazole and ciprofloxacin) at 0.40 mg/L in chitosan fibrin hydrogel

drying the canals, double antibiotic paste was placed using lentulospiral and left for 4 weeks.

At the subsequent appointment, revascularization was considered if asymptomatic. Under isolation, after irrigating with 10 ml of sterile saline and 10 ml of 17% EDTA, and subsequent drying, a sterile #20 k-file was inserted 2 mm beyond the determined working length to stimulate bleeding for clot initiation [Figure 5b]. A moist cotton pellet was placed for 10 min to allow clot formation. CollacoteTM was placed if necessary, to achieve hemostasis.

In the PRF group, patient's venous blood was centrifuged at 2700 rpm for 14 min to prepare the scaffold. Subsequently, A-PRF was carefully extracted using PRF instruments and inserted into the canal with a hand plugger.

For the three hydrogel groups, the hydrogels initially viscous fluids at the room temperature were preloaded into syringes for placement in the apical third of the canal over the blood clot [Figure 4]. One milliliters of the hydrogel liquid was placed in the canal [Figure 5c]. After allowing 7 min for gelation, a hand plugger was used to push the scaffold to the apical third.

Then, the access cavity was sealed with a 3–4 mm thick layer of biodentine (Septodont, France) [Figure 5d] and finally restored with glass ionomer cement [Figure 5e] and composite resin [Figure 5f]. All intraoral procedures were done using loupes with ×3.5 magnification. The patient was kept on recall during the period of study at 3, 6, and 12 months, in case of treatment failure MTA apexification would be carried out.

Imaging methods, blinding and evaluation

Standardized Intra-Oral PeriApical (IOPA) X-rays were recorded using extension cone paralleling (XCP) positioner (Dentsply) with a 0.8 mm tube X-ray machine and E-film, following the parallel radiographic technique. All films underwent processing in an automatic processor. IOPA X-rays were utilized for the interim analysis over the 12-month period.

Cone-beam computed tomography (CBCT) images were captured using the NewTom Giano HR Professional (NewTom Mfg Corp) with a voxel size of 0.300 mm. Images were analyzed using specific software (NNT software) on a PC work station running Microsoft Windows XP (Microsoft Corp, Redmond, WA). All digital images were assessed by two pretrained and blinded observers (all the scaffolds were radiolucent and thus unidentifiable during CBCT evaluation) and Kappa statistics were used to check inter-observer agreement. Patients were also blinded to mitigate any potential bias.

Primary outcome measures

Quantitative measurements of changes in size of the apex,

increase in root length, increase in dentin thickness, and periapical healing parameters were conducted using the NNT software at 12 months.

The method involved determining the most prominent changes in root length, dentin thickness, and apical diameter. This was achieved by selecting the maximum length observed in sagittal sections for root length, identifying the maximum dentin thickness in coronal sections for dentin thickness, and measuring the minimum diameter of apical foramen within axial sections of the apical third [Table 1a].

CBCT Periapical Index (PAI) scoring followed the methodology outlined by Estrela *et al*.[13] Radiolucent areas suggesting periapical lesions were assessed using the software's tools in three sections: sagittal, coronal, and axial. The CBCT PAI score was determined by the largest extent of the lesion, utilizing the following scoring system [Table 1b] - (a six-point scoring, ranging from 0 to 5).

- 0 - Intact periapical bone structures
- 1 - Periapical radiolucency size: 0.5–1 mm
- 2 - Periapical radiolucency size: 1–2 mm
- 3 - Periapical radiolucency size: 2–4 mm
- 4 - Periapical radiolucency size: 4–8 mm
- 5 - Periapical radiolucency size: 8 mm
- 6 - (n) + E-Expansion of periapical cortical bone.

(n) + D-Destruction of periapical cortical bone.

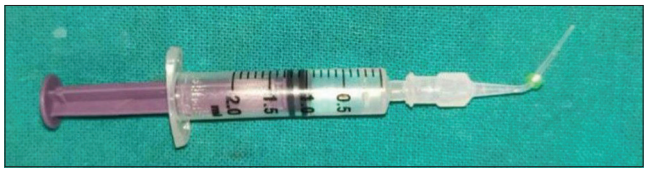


Figure 4: Premeasured dose of injectable fibrin hydrogel in syringe

Secondary outcome measures

The presence or absence of any clinical signs and symptoms of apical periodontitis or persistent infection were the secondary outcome measures.

Statistical analysis

The data were input and organized in MS Excel, and then analyzed statistically utilizing the SPSS software (IBM SPSS Statistics, New York, USA) software version 2.1. A significance level of 5% was established. The data underwent evaluation for normal distribution through the Shapiro–Wilk test and statistical analysis was applied accordingly. All the significance tests used have been mentioned.

OBSERVATIONS

Out of the 56 patients, one from each group was lost to follow-up. Clinically and radiographically, all 52 teeth in four groups (13/group) received the intended treatment based on the random allocation. All showed excellent results throughout the study. Patients were completely asymptomatic and presented clinical signs of healing [Table 2a]. There were no adverse effects seen in any of the patients.

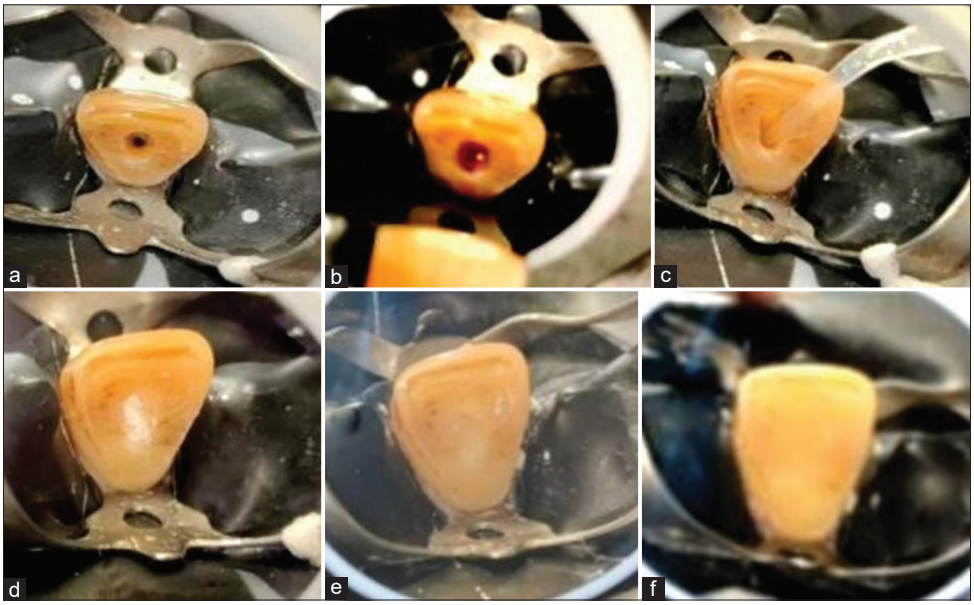
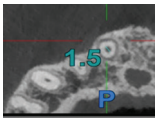
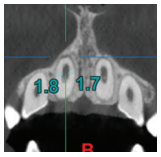
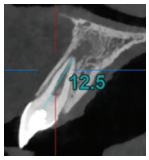
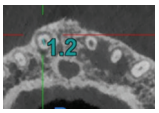
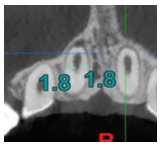
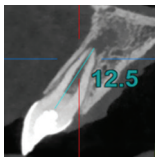
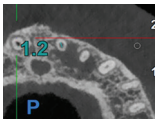
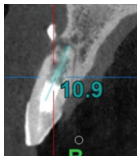
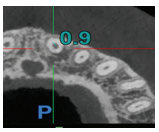
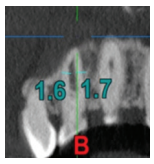
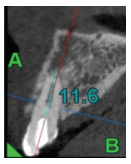
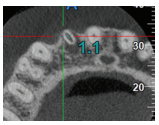
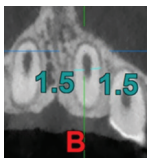
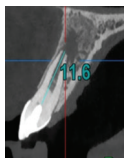
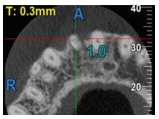
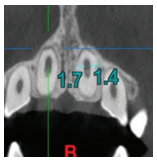
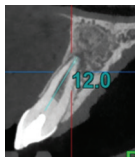
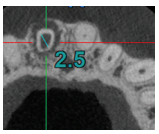
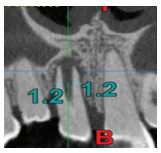
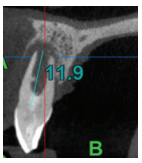
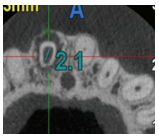
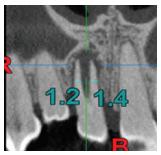
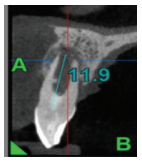


Figure 5: (a) Access opening under rubber dam isolation. (b) Inducing bleeding inside the canal. (c) Placement of scaffold over the clot using syringe. (d) Placement of biodentin over the scaffold at the level of Cemento-Enamel Junction (CEJ). (e) Placement of Glass Ionomer Cement (GIC). (f) Placement of composite filling for coronal seal

Table 1a : Comparative Quantitative analysis using CBCT images for change in root length, root dentin thickness and apical closure at end of 12 months for groups 1,2,3 and 4

Group/Parameter	Apical closure	Radicular dentin thickness	Root Length	Before/After 12 months
Group 1: PRF				Before
				After
Group 2: DA + Chitosan Hydrogel				Before
				After
Group 3: DA-Hydrogel Group				Before
				After
Group 4: Chitosan Group				Before
				After

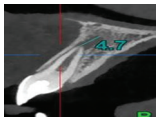
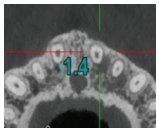
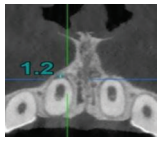
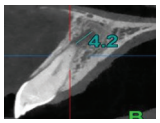
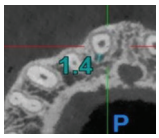
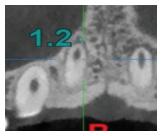
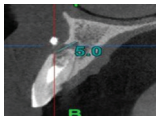
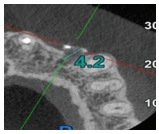
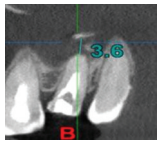
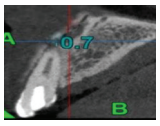
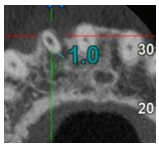
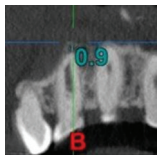
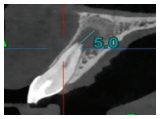
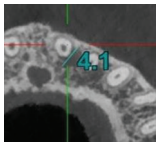
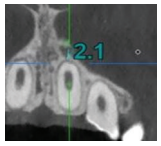
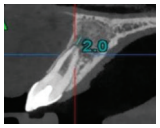
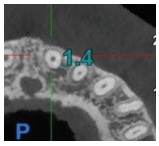
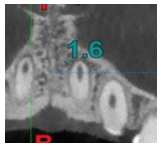
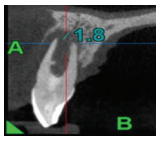
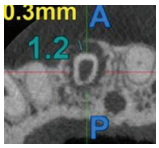
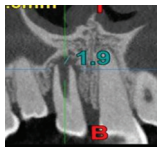
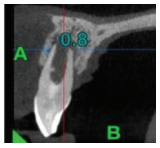
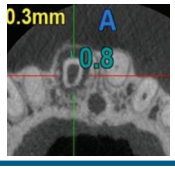
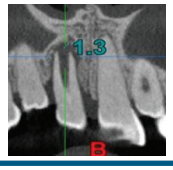
Percentage change in root length

Among all the three groups, Group 2 had the highest percentage increase in root length 37.72%, followed by Groups 3, 4, and Group 1 with means of 27.20%, 20.98%, and 20.95%, respectively.(Kruskal–Wallis test, $P = 0.004$). In Kruskal–Wallis pair-wise test ($P < 0.05$), there was significant difference between Group 2 and Group 1 and Group 2 and Group 4 (2 > 1, 2 > 4) [Table 2a].

Percentage change in apical diameter

The mean percentage apical closure in Groups 1, 2, 3, and 4 was 2.83%, 9.61%, 4.47%, and 4.35%, respectively [Table 1a]. Group 2 showed highest percentage change in apical diameter, and the results were statistically significant ($P = 0.05$) (one-way ANOVA). *Post hoc* Tukey's analysis showed that Group 2 had significantly better results than both Groups 1 and 4.($P < 0.05$).(2 > 1, 2 > 4) [Table 2a].

Table 1b : Comparative Qualitative analysis using CBCT images for periapical healing at end of 12 months for groups 1,2, 3 and 4

Group/Parameter	Sagittal Section	Axial Section	Coronal section	Before/After 12 months
Group 1: PRF				Before
				After
Group 2: DA + Chitosan Hydrogel				Before
				After
Group 3: DA-Hydrogel Group				Before
				After
Group 4: Chitosan Group				Before
				After

Percentage change in radicular dentinal wall thickness

(Kruskal–Wallis test, $P = 0.008$) The mean percentage dentinal wall thickening in Groups 1, 2, 3, and 4 was 9.82%, 27.86%, 20.43%, and 11.17%, respectively [Table 1a]. Group 2 showed highest percentage change in radicular dentine thickness, and the results were statistically significant (Kruskal–Wallis Pair-wise test, $P < 0.05$) ($P < 0.05$).($2 > 1, 2 > 4$) [Table 2a].

Periapical healing

As the PAI scores showed a normal distribution, for intragroup evaluation of preoperative and post-operative PAI scores, Paired *t*-test is used. For intergroup evaluation of PAI scores before and after procedure one-way ANOVA test is used.

In this study, best result was observed in double antibiotic-loaded Chitosan fibin hydrogel, where about 92.3% of the cases showed excellent periapical healing. This was followed by double antibiotic-loaded fibrin hydrogel with 83.33% periapical healing, PRF with healing percentage of 77%, and least being chitosan fibrin hydrogel with 69.3% periapical healing [Table 2b].

After evaluating all four parameters, the research hypothesis was supported, and the null hypothesis

was refuted ($P < 0.05$). The group treated with double antibiotic-loaded Chitosan fibin hydrogel demonstrated superior outcomes compared to the PRF group concerning root length augmentation, apical closure, and dentinal wall thickening. However, there was no notable distinction observed in terms of periapical healing.

DISCUSSION

REP focus on cell homing/guiding of stem cells of the apical region in teeth with necrotic pulp and apical periodontitis. REP offers benefits including high healing rates, continued root development, increased crown-root ratio, and apical closure. Successful REP depends on factors such as proper disinfection, suitable scaffold for stem cell growth, and coronal seal to prevent reinfection.

In this present study, immature teeth with necrotic pulps also had the presence of periapical lesion in all the cases, which healed over the duration of the study. This shows the effectiveness of the new scaffolds and the REP in general in the management of such cases. The primary outcome measures of the study are the indicators of continued root development and the secondary outcome measures the healing of periapical lesion and the eradication of related infection.

Random allocation, double-blinding large sample size, positive control group (PRF group), primary and secondary outcome measures, and high adherence to the protocol are the strengths of this clinical study.

If we consider all regenerative parameters used in this study, it is quite clear from results we obtained, that double antibiotic-loaded chitosan fibrin hydrogel was superior among all scaffolds used. This can be explained with the delivery of antibiotic drugs ciprofloxacin and metronidazole, along with providing a meshwork for the formation of blood clot which acts as a source for release of TGF-β that stimulates the odontogenic differentiation of human dental pulp cells (HDPCs).[14] Fibrin meshwork also leads to more efficient cell migration and proliferation.[15]

Physical characteristics of fibrin scaffolds such as gelation, viscosity, and elasticity can be regulated through their fabrication procedures along with incorporation of antibacterial agents.[16]

SEM study shows a meshwork structure that has pores of size ranging from 20 to 40 μ [Figure 2a-c]. Thus, the meshwork can release the antibiotic molecules from these and house platelets (~2 μ) easily on clotting of blood.

Chitosan is a natural polysaccharide formed by N-deacetylation of chitin. It has antimicrobial activity against both Gram-negative and positive bacteria, including *Enterococcus fæcalis*.[17,18] This can be attributed to its positive charge on the particle surface that binds to the negatively charged bacterial cell wall and also the ability to bind to bacterial DNA.[19] Antibiotic-loaded chitosan hydrogels have previously showed good results in the applications in the medical field.[20]

The release of metronidazole and ciprofloxacin from double antibiotic-loaded chitosan fibrin gel exhibited a linear pattern over 7 days, contrasting with the initial burst release (70%) followed by continuous slow release observed with antibiotic fibrin hydrogel [Figure 3b]. The chitosan particles have ability to enhance the bioavailability of drugs and act as controlled drug delivery systems. It can sustain the antibiotic release. This is due to molecular level interaction between the

Table 2a: Comparative quantitative analysis of change in root length, root dentin thickness, and apical closure at the end of 12 months for Groups 1, 2, 3, and 4

Group	Percentage change in apical closure		Percentage increase in root length		Percentage increase in root dentin thickness	
	Mean±SD	ANOVA test	Mean±SD	Kruskal–Wallis test	Mean±SD	Kruskal–Wallis test
Group 1: PRF	2.83±2.55	P^*=0.05 (S)	20.95±13.33	P^*=0.004(S)	9.82±9.49	P^*=0.008 (S)
Group 2: DA + chitosan hydrogel	9.61±5.60	*Post hoc* analysis:	37.72±9.23	Kruskal–Wallis	27.86±13.03	Kruskal–Wallis
Group 3: DA−hydrogel group	4.47±3.20	2>1 (0.008)	27.20±15.62	pairwise: 2>1 (0.003)	20.43±21.98	pairwise: 2>1 (0.001)
Group 4: Chitosan group	4.35±4.19	2>4 (0.008)	20.98±11.90	2>4 (0.038)	11.17±10.27	2>4 (0.036)

*The mean difference is significant at the 0.05 level. S: Significant ($P<0.05$), NS: Nonsignificant ($P\geq0.05$). SD: Standard deviation, PRF: Platelet-rich fibrin, DA: Double antibiotic

Table 2b: Comparative qualitative analysis of periapical healing at the end of 12 months for Groups 1, 2, 3, and 4

Groups	Preoperative score						Postoperative score						Paired *t*-test
	0, *n* (%)	1, *n* (%)	2, *n* (%)	3, *n* (%)	4, *n* (%)	5, *n* (%)	0	1, *n* (%)	2, *n* (%)	3, *n* (%)	4, *n* (%)	5, *n* (%)	
Group 1 (13)	0	0	2 (15.4)	2 (15.4)	4 (30.8)	5 (38.5)	0	6 (46.2)	4 (30.8)	1 (7.7)	0	0	P<0.001 (significant)
Group 2 (13)	0	0	0	4 (30.8)	1 (7.7)	8 (61.6)	0	9 (69.2)	3 (23.1)	1 (7.7)	0	0	P<0.001 (significant)
Group 3 (13)	0	0	2 (15.4)	7 (53.9)	1 (7.7)	3 (23.1)	0	8 (61.6)	4 (30.8)	1 (7.7)	0	0	P<0.001 (significant)
Group 4 (13)	0	0	2 (15.4)	3 (23.1)	2 (15.4)	6 (46.2)	0	7 (53.9)	2 (15.4)	0	0	0	P<0.001 (significant)
One-way ANOVA			P=0.149 (NS)						P=0.402 (NS)				

NS: Nonsignificant

antibiotics such as ciprofloxacin and metronidazole and chitosan that occurs due to the charged surface of the chitosan particles.[21,22] The release of ciprofloxacin and metronidazole can thus be divided into two phases: a rapid release phase facilitating rapid sterilization initially and a slow-sustained release phase that provides a long term antibacterial effect thereafter.[10,23]

One milliliter of premeasured scaffolds was loaded in syringes for placement based on the release graphs, so as to achieve 1 mg/L concentrations of metronidazole and ciprofloxacin by 24 h. This concentration is effective in eradication of persistent bacteria like *Enterococcus faecalis* without any deleterious effect on SCAP.[24]

Ducret *et al*.[8] pointed that 0.5% chitosan addition can impart an antimicrobial action to fibrin hydrogel, which is effective against planktonic *E. faecalis* without having any effect on Dental pulp stem cells (DPSC).

Upon evaluating the gelation time of 0.4% chitosan–fibrin formulation at 37°C, it was found to convert into a gel within 7 ± 2 min, thus providing sufficient time for slow injection into the canal space of single to three-rooted teeth in a clinical scenario. Consequently, a 0.4% chitosan concentration was chosen.

Interestingly, both the antibiotic-releasing hydrogel groups stimulated the periapical healing, apical closure, root length formation, and root dentin thickening. It can be concluded from these observations that, the fibrin meshwork releasing antibiotics itself plays a substantial role in odontogenic differentiation by providing a microbe-free environment. Addition of chitosan can synergize and prolong this effect and thus improve the results even further.

Future trials with longer follow-up of the scaffolds used in this study can improve the understanding of their potential. Similarly, other growth factors and drugs can be loaded with the present hydrogel to study the effect on REP.

Limitations of the study

Follow-up of 1 year only is not enough for properties such as pulp sensitivity that can take minimum of 14–16 months to develop. It may take upto 4 years or even more for complete healing of large apical periodontitis lesion.

Since, the current study was an interventional study, thus the operator could not be blinded to the treatment method; however, randomization and allocation blinding was done and treatment evaluation was conducted by two-blinded experienced dentists.

CONCLUSION

Within the limitations of this study, all groups showed satisfactory outcomes. The double antibiotic-loaded chitosan fibrin hydrogel excelled in promoting apical closure, root lengthening, and dentinal wall thickening. The difference between the double antibiotic-loaded chitosan fibrin hydrogel and both PRF and chitosan-loaded fibrin hydrogel was statistically significant. However, its disparity with the double antibiotic-loaded fibrin hydrogel was not. Periapical healing was good across all scaffolds, with no significant differences observed.

Extended clinical research is needed for a comprehensive understanding of the potential of these scaffolds in regenerative endodontics.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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