CASE REPORT

REP IN THE MANAGEMENT OF NON VITAL UPPER PERMANENT MATURE TEETH WITH PERIAPICAL PATHOLOGY USING BLOOD CLOT & PRP AS SCAFFOLD : A CASE SERIES

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ABSTRACT

Interest in attempting Regenerative Endodontic Procedures (REPs) in nonvital mature permanent teeth with or without periapical pathology, emerged from its success in necrotic immature permanent teeth. The present case series describes the management of six such teeth with apical pathology using REP with Platelet Rich Plasma (PRP) or Blood Clot (BC) as a scaffold. Methods: After gaining access, root canals were cleaned & irrigated with 1.5% NaOCL & saline, filled with $Ca(OH)_2$ and sealed with cavit. On 2nd appointment irrigation was done with 1.5% NaOCl followed by 17% EDTA, finally with normal saline. Autologous PRP was injected into three teeth and fresh bleeding was induced within the root canals of rest under LA without vasoconstrictor. After placing 3 mm of MTA teeth were restored with composite . Follow up visits were done for clinical & radiological assessment using both IOPAR & CBCT. Result: Teeth were asymptomatic, functioning normally showing evidences of bone healing of apical radiolucencies. Positive pulp sensibility response could not be elicited. Conclusion: REP can be undertaken in mature nonvital permanent tooth with apical pathology using PRP/BC as scaffold.

KEY WORDS

REPs, Blood Clot, PRP, CBCT

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INTRODUCTION

The encouraging results of regenerative endodontics in immature permanent teeth had lead to an immense interest for Regenerative Endodontic Procedure (REP) in nonvital mature permanent teeth.

Since earlier works by Ostby[1] and Nygaard-Ostby and Hjortdal[2], there have been numerous attempts to revitalize teeth with valuable changes in the procedural protocol of REP[3]. Aim is to regenerate the pulp-dentin complex with restoration of odontoblastic layer lining the dentin surface, and establishing the micro neurovasculature in root canal system for root maturation and regaining vitality of the tooth along with wound healing within the bone through regeneration if there is any.

Reconstitution of the neurovascular system in root canals will provide pulp tissues with an immune system, which will function as the first line of defence against microbial challenge. The gain of nerve function in regenerated pulp tissues will provide an alarm system during the tissue injury and protect the pulp from further damage. In addition to pulp regeneration, dentin may be deposited along the root canal walls that had been lost during mechanical instrumentation[4], although the dentin deposition has not been consistently demonstrated[5].

REP in mature teeth will likely to encounter more challenges than in immature teeth. Availability of less stem/progenitor cells as age advances and narrower apical pathways may create hindrance for migration of these cells together with greater difficulty in disinfecting root canals in mature teeth. Search literature does not reveal sufficient successful attempts of REP in mature permanent teeth with necrotic pulp. The present case series reports management of nonvital upper permanent anterior teeth with apical periodontitis through REP.

CASE SERIES

Patients from OPD of Dr. RADC & H were selected who complained of pain and swelling in upper anterior region having history of trauma and non-contributory medical history. After thorough

clinical examination, pulp sensibility tests were conducted with cold (Endo frost, Coltene) and Electric Pulp Test (EPT) to confirm that the teeth were non vital. Teeth were evaluated radiographically. Teeth with immature roots were excluded. Three patients were included in the study. They were informed about the procedure and consent was taken. There after clearance from the institutional ethics committee was taken. Platelet Rich Plasma (PRP) in 3 teeth in 1 patient and Blood Clot (BC) in 3 teeth in 2 patients were used as scaffold for REP. During the procedure apical enlargement of root ends to at least upto #30 was ensured. After the procedure, patients were recalled for clinical and radiological evaluation on 3rd & 6th month then after every 6 months interval. However the patients in the present case series could not attend all the scheduled followup visits because of the COVID pandemic situation.

In addition to IOPAR, quantitative CBCT evaluation was done with "Sky view 3D Panoramic Imager" (My-Ray Dental Imaging, Imola, Italy). All the pre and postop scanned samples were imported into Irys viewer software Version 6.2 (MyRay CBCT) to measure the area of periapical radiolucent area in mm2 and its bone density (i.e mean Gray value) expressed in mean Hounsfield unit (Hu).These measurements were made in three planes- axial, sagittal & coronal[6]

Case 1:

Introduction:

IOPAR of discolored 21 and slightly mobile 22 of a 35 year old female patient, revealed periapical pathology involving 21,22,23 with attempted Root Canal Therapy (RCT). (Fig. 1- A) few years ago. Pathology was evaluated through CBCT (Fig.1-B).

Methods:

Under Local anaesthesia (2% lidocaine with 1:80,000 epinephrine) and rubber dam application teeth were isolated. Access cavities were modified (Fig.1-C), working lengths were determined after removal of gutta-percha from 23(Fig. 1-D). After cleaning and shaping, irrigation was done with copious amount of 1.5% NaOCl. Canals were dried with paper points. Calcium hydroxide (Ultracal XS, Ultradent, USA) was used as intra canal medicament and the access cavities were closed with cavit (3M ESPE) (Fig.1-E & Fig.1-F).

Patient was advised to report after 4 weeks or whenever any problem arises.

On second visit under rubber dam application temporary restoration was removed. Normal saline was used for flushing out Ca(OH)2, followed by irrigation with 5 ml of 1.5 % NaOCl and 20 ml of 17% EDTA for a period of 5 minutes each.

Then 10 ml of blood was drawn from patient's antecubital vein (Fig.1-G). It was transferred to test tube containing 1ml heparin to be centrifuged (centrifuge machine : REMI R-8C) at 1500 rpm (400 g, 8 cm radius of centrifugation) for 5 minute (Fig.1-H). Test tube was taken out. Plasma was separated from RBC with buffy coat in between (Fig.1-I). Plasma and buffy coat was transferred into another test tube which was subjected to second spin in the same machine at 2700 rpm for 17 minutes. In this test tube PRP was seen distinctly separated from PPP (Fig.1-J) at the top which was thrown away and the PRP was taken in another test tube (Fig.1-K).1 ml of CaCl2 was added into it and then the PRP was transferred into a insulin syringe to be injected into the canals of 21, 22 & 23 (Fig.1-L) after drying with paper points. Waiting 10- 15 minutes for its clotting (Fig.1-M) collaplug (SRI GOPAL KRISHNA LABS PVT. LTD. MUMBAI) was placed over it for application of 3mm of MTA (Angelus) below CEJ (Fig.1-N). Waiting for a period of 15-20 minutes as its setting time, teeth were restored with composite resin (Z350 Xt 3M ESPE Filtek) (Fig.1-O)

Patient came on follow up visit after 12 month and 18 month (Fig.1.-P to Fig.1- S)

On follow up visits the patient was asymptomatic and the teeth were functioning normally. The radiographic evaluations revealed definite evidence of healing of the periapical pathology both in IOPAR and CBCT (Table 1) But pulp sensibility test was -ve.

Case 2:

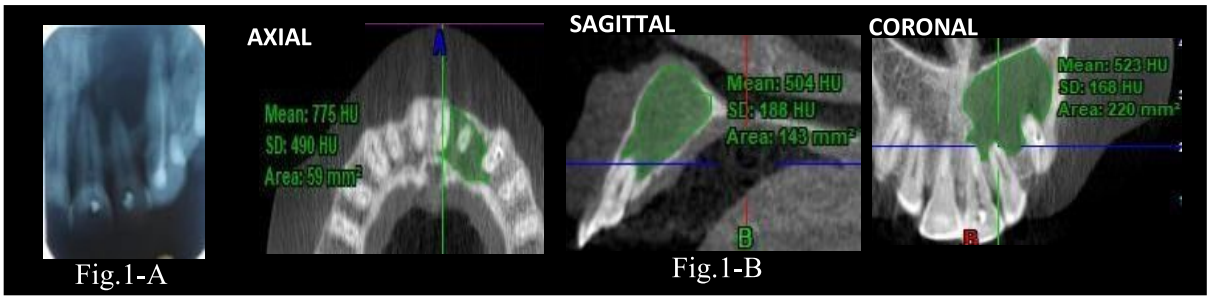
Introduction:

After evaluation (Fig.2-A & Fig.2-B) a 20 year old male patient was diagnosed with acute apical periodontitis in relation to nonvital 21 having pain on percussion and palpation. It was planned to undertake REP taking autologous Blood clot as scaffold.

Methods:

Under Local anaesthesia (2% lidocaine with 1:80,000 epinephrine) and rubber dam application access cavity was prepared (Fig.2-C), WL measurement was done (Fig.2-D) After thorough cleaning, shaping and irrigation procedure Ca(OH)2 (Ultracal XS, Ultradent, USA) was placed within the canal as intracanal medicament (Fig.2-E) which was followed by placement of Cavit (3M ESPE) as temporary restoration. Patient was disposed for 4 weeks.

On second appointment under Local anaesthesia (2% lidocaine without epinephrine) and rubber dam isolation cavit was removed, the calcium hydroxide was flushed out with normal saline(Fig.2-F). 5 ml of 1.5% NaOCl and 20 ml of 17% EDTA was used as irrigating agent for a period of 5 minutes each which was followed by final irrigation with normal saline.



Preop: Fig.1-A IOPA-R ; Fig.1-B CBCT: Axial, Sagittal &Coronal view

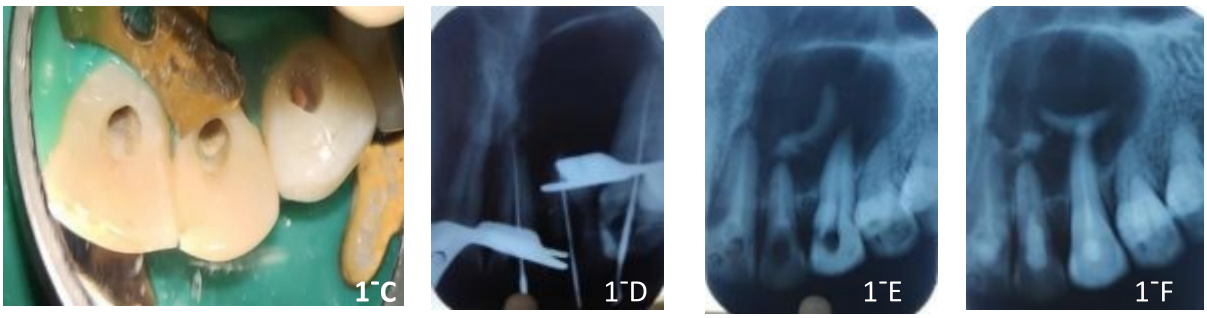


Fig.1-C Access cavity modified Fig.1-D WL determined Fig.1-E Ca(OH)2 placed in 21,22 Fig.1-F Closed with cavit after placing Ca(OH)2

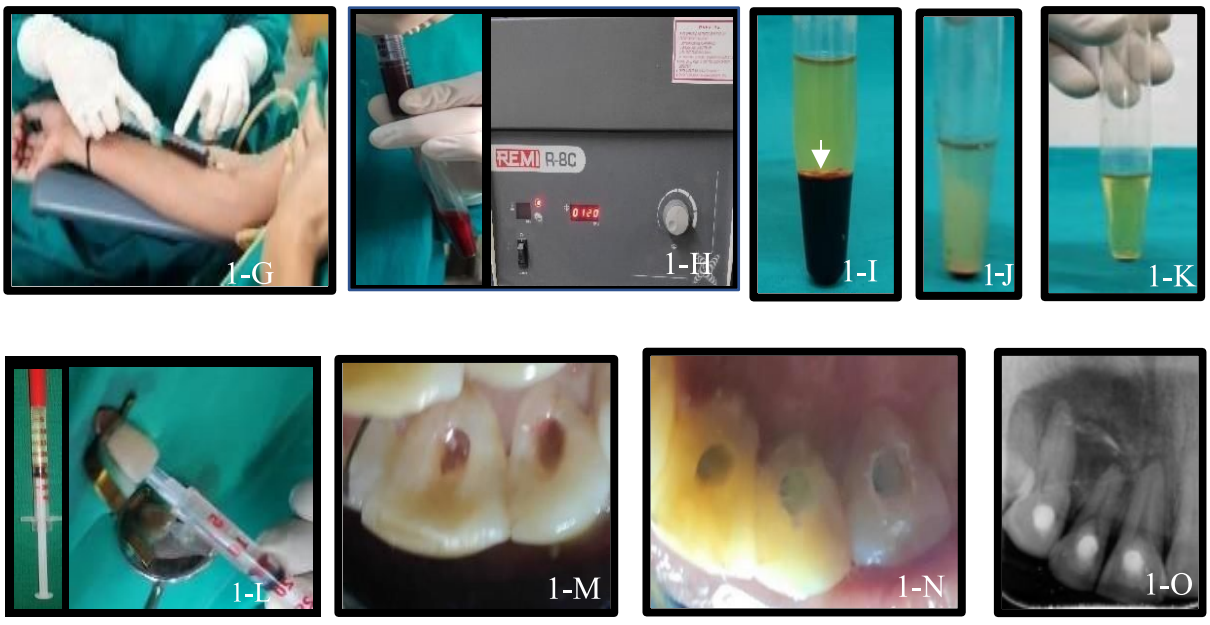
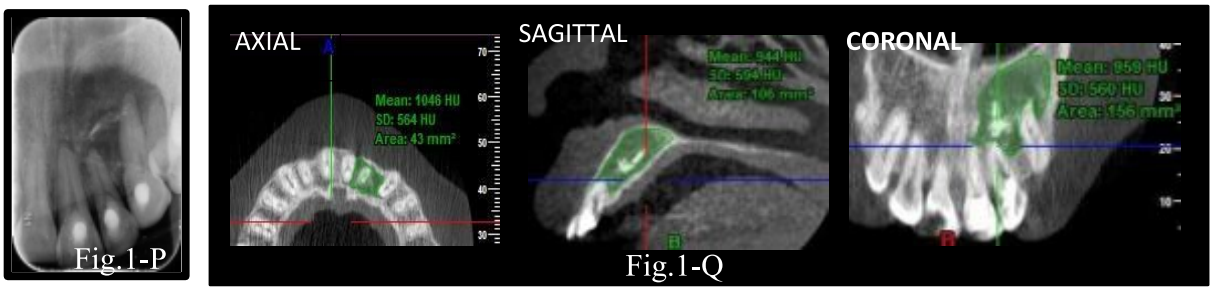
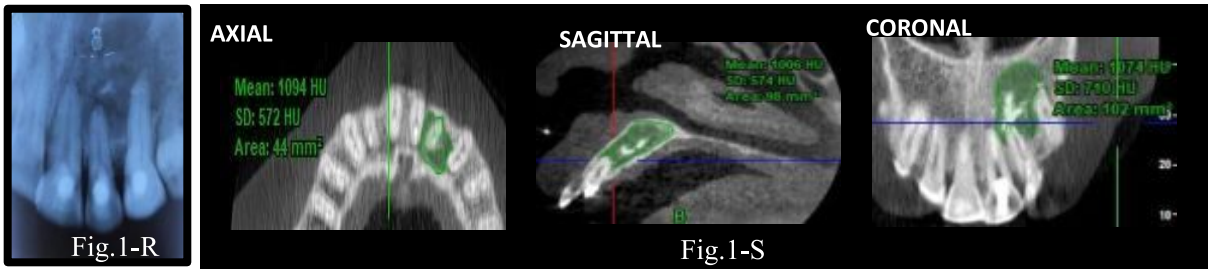


Fig.1-G: Blood drawn from antecubital Vein. Fig.1-H: Transferred to test tube containing heparin to be placed in centrifuge machine(REMI-R8C). Fig.1-I: Plasma separated from RBC with buffy coat in between. Fig.1-J:PPP separated at the top. Fig.1-K: PRP was taken into another test tube into which 1 ml of calcium chloride was added. Fig.1-L:Freshly Prepared PRP was taken into an insulin syringe and injected into the canal. Fig.1.M: PRP clot after 10-15 minutes. Fig.1-N: MTA upto CEJ. Fig.1-O: Post op IOPAR after composite resin restoration.

Patient came on follow up visit after 12 month and 18 month (Fig.1.-P to Fig.1- S)



12 MONTH FOLLOWUP: Fig.1-P: IOPAR, Fig.1-Q CBCT showing increase in Hu & decrease in area of lesion



18 MONTH FOLLOWUP: Fig.1-R: IOPAR ,Fig.1-S CBCT showing further increase in Hu & decrease in area of lesion

Table 1; Bone density expressed in Grey Value(GV) & reduction of Surface Area (A) of bony crypt in respect of Case 1

TIME PERIOD		CBCT				%Red of A= ↓ A/ Preop (av)×100	% Gain in GV
		AXIAL	SAGITTAL	CORONAL	AVERAGE		
Preop	GV	775	504	523	600.6		
	SA	59	143	220	140.6		
12 mon.	GV	1046	944	959	983		63.6
	SA	43	106	156	101.6	27.7	
18 mon.	GV	1094	1006	1074	1058		76.1
	SA	44	98	102	81.3	42.1	

The canal was then dried with paper points and intracanal bleeding (Fig.2-G) was induced upto CEJ from periapex using precurved #20 hand instrument. After 10-15 minutes following clot formation the collaplug was placed over it for placement of 3 mm of MTA (Fig.2-H) followed by restoration with the composite resin (Fig.2-I) as was done in case 1.

On 3 & 6 month recalled visits he was asymptomatic and the tooth was functioning normally.

Radiologically there was evidence of healing of apical pathology (Fig.2-J to Fig.2-M)

Case 3:

Introduction:

18 year old male patient had fractured incisal enamel of both 21 & 22 which were also tender on palpation and percussion. Radiologically there was apical pathology in relation to

Methods:

Similar to the previous two cases, under Local anaesth3e-sBia and rubber dam application both the teeth were accessed (Fig.3-C), cleaned after WL

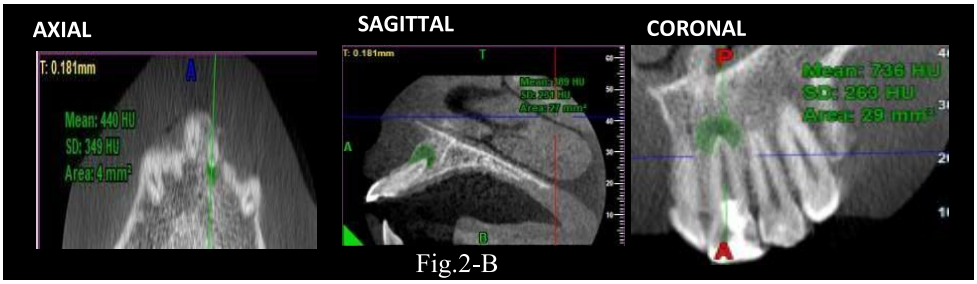
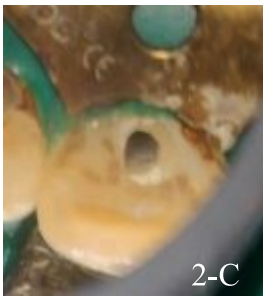


Fig.2-A

Fig.2-B

Preop: Fig.2-A IOPA-R ; Fig.2-B CBCT: Axial, Sagittal &Coronal view



2-C

2-D

2-E

Fig.2-C Access cavity prepared Fig.2-D WL taken Fig.2-E Ca(OH)2 placed

2-F

2-G

2-H

2-I

Fig.2-F Ca(OH)2 removed. Fig.2-G Blood clot induced. Fig.2-H MTA placed.
Fig.2-I IOPA Xray after composite restoration

Fig.2-J

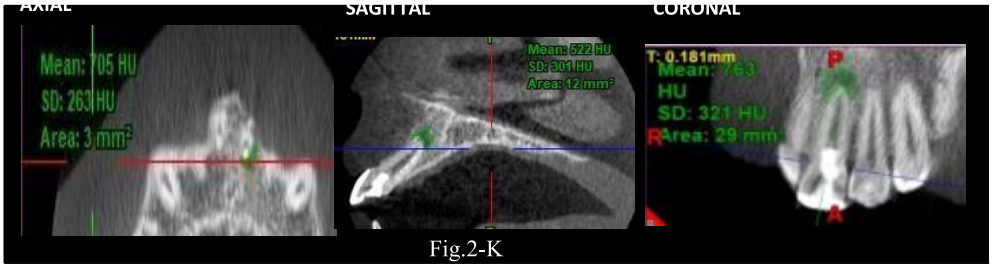


Fig.2-K

3 MONTH FOLLOWUP: Fig.2-J: IOPAR, Fig.2-K: CBCT showing increase in Hu & decrease in area of lesion

Fig.2-L

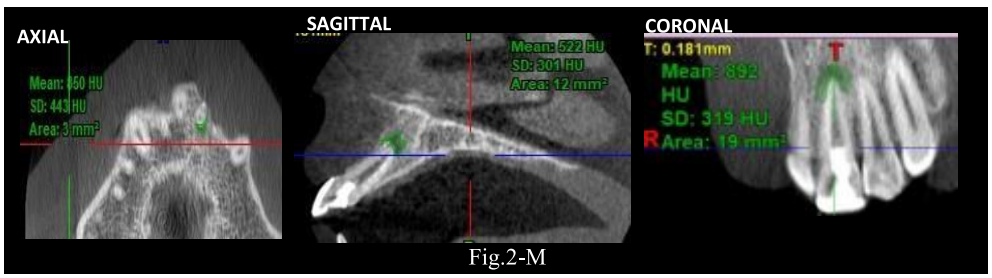


Fig.2-M

6 MONTH FOLLOWUP: Fig.2-L: IOPAR Fig.2-M: CBCT showing further increase in Hu & decrease in area of lesion

RESULTS:

Table 2; Bone density expressed in Grey Value (GV) & reduction of Surface Area(A) of bony crypt in respect of Case 2

TIME PERIOD		CBCT				%Red of A= ↓A/ Preop (av)×100	% Gain in GV
		AXIAL	SAGITTAL	CORONAL	AVERAGE		
Preop	GV	440	389	736	521.6		
	SA	4	27	29	20		
3 mon.	GV	705	522	763	663.3		27.1
	SA	3	12	29	14.6	27	
6 mon.	GV	850	724	892	822		57.5
	SA	3	4	19	8.6	57	

measurement (Fig.3-D) shaped, irrigated and calcium hydroxide given as intracanal medicament (Fig.3-E)

Then on 2nd visit, after removal of Ca(OH)2 (Fig.3-F) and following proper irrigation, intracanal bleeding (Fig.3-G) was induced in the same way as it was done in case 2. On clotting of intracanal blood & placement of the collaplug, 3 mm of MTA was placed (Fig.3-H) and (Fig.3-I). After its setting in 15-20 minutes, teeth were restored with composite resin in the same way as it was done previously. (Fig.3-J)

On follow up visits at 3rd (Fig.3-K & Fig.3-L) and 6th month (Fig.3-M & Fig.3-N) the patient was seen to be asymptomatic and radiologically the apical pathology was seen in the process of healing.

DISCUSSION

The idea of developing new vasculature within the root canal system through REP instead of performing root canal therapy in nonvital mature permanent tooth with apical periodontitis is novel. Absence of clinical symptoms like pain, swelling and bringing the teeth back to function with gradual reduction of periapical pathologies in the present case series are indicative of success towards REP.

Disinfection procedure of root canal system followed here and intracanal medication used has been proven in literature[7,8]. Efficacy of BC9 & PRP9 as scaffold in REP is also documented.Instead of Platelet Rich Fibrin (PRF), another commonly used Autologous Platelet Concentrate as scaffold, PRP was used because it can be easily introduced into root canal system via insulin syringe.

Apical enlargement atleast upto #30 was ensured in the present case series. This facilitates progenitor cell migration within the root canal from periapex10 PRP contains number of mitogenic growth factors such as Platelet Derived Growth Factor (PDGF), Transforming Growth Factor- β (TGF- β), Epidermal Growth Factor (EGF), Vascular Endothelial Growth Factor (VEGF) which are important for angiogenesis and improved tissue vascularization[8]. These are held by the fibre mesh developed by polymerization of fibrinogen. As a result of double centrifugation process the mesh which is formed is non elastic and have bilateral junction (Condensed Tetramolecular) of the fibrin11 and releases these biomolecules slowly. 80 % TGF β 1 and similar level of total PDGF-AB are released in first day and contribute to wound healing8.

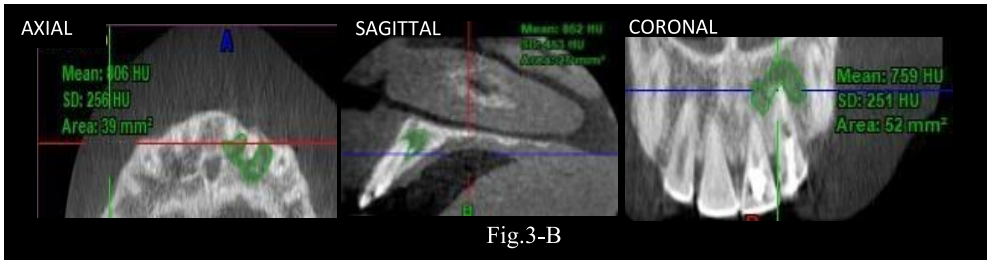
There were presence of wounds within the bone in the periapex of the teeth under study which healed gradually. Strong evidence of gradual healing was observed in IOPAR as well as quantitatively in CBCT scans.

Ease of operation with minimum armamentarium and no extra cost draws attention of using BC. But it is difficult to obtain bleeding upto CEJ at times.

Within the followup periods apart from patient being asymptomatic clinically, radiological evaluations revealed definite evidence of wound healing periapex of teeth treated both by BC & PRP mediated REP in present case series.

MTA- a Bioactive materials is recommended as capping material over scaffold[3].

MTA(Angelus) used here sets within clinically acceptable period of time. The desired leak proof seal was obtained dually from this material & micromechanical bonding of composite with surface enamel of access cavity. Pulp sensibility test was negative may be because of presence of thick MTA at coronal part of root canals[12].Though there is evidence of deposition of dentin along the instrumented root canal wall[13], histopathological study fails to reveal such well organized dentinal tubules in respond to pulp sensibility test in the same way as it is done by natural tissue[14], but with passage of time different response may be elicited.



Preop: Fig.3-A IOPA-R ; Fig.3-B CBCT: Axial, Sagittal &Coronal view

Fig.3-C After access opening Fig.3-D WL measurement, Fig.3-E Ca(OH)2 placed

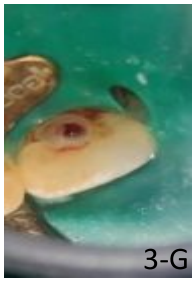
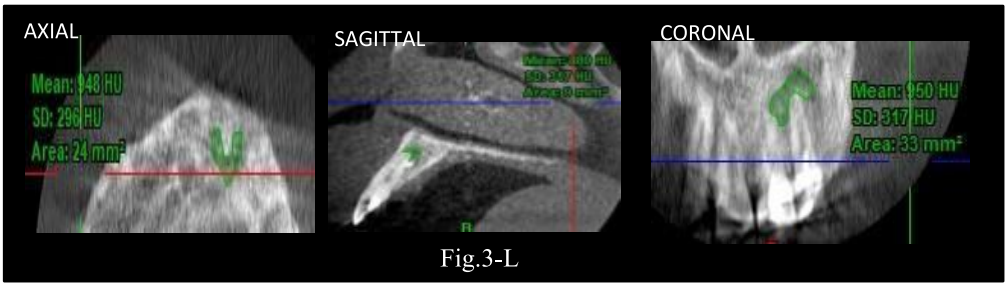
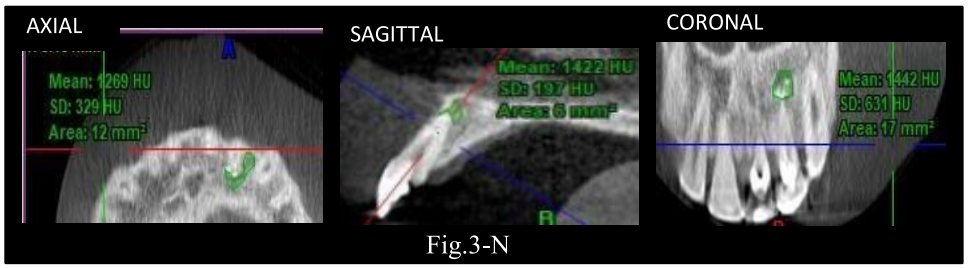


Fig. 3-F Ca(OH)2 removed on 2nd visit. Fig. 3-G After proper irrigation & drying the canal blood clot induced. Fig. 3-H & Fig. 3-I MTA placed upto CEJ over the clot & collaplug Fig. 3-J IOPA showing composite restoration



3 MONTH FOLLOWUP : Fig. 3-K: IOPAR , Fig.3-L: CBCT showing increase in Hu & decrease in area of lesion



6 MONTH FOLLOWUP: Fig.3-M: IOPAR Fig.3-N: CBCT showing further increase in Hu & decrease in area of lesion

Table 3; Bone density expressed in Grey Value(GV) & reduction of Surface Area(A) of bony crypt in respect of Case 3

TIME PERIOD		CBCT				%Red of A= A/ Preop (av)×100	% Gain in GV
		AXIAL	SAGITTAL	CORONAL	AVERAGE		
Preop	GV	806	852	759	805.6		
	SA	39	25	52	38.6		
3 mon.	GV	948	889	950	929		15.3
	SA	24	6	33	21	45.5	
6 mon.	GV	1269	1422	1442	1377.6		71
	SA	12	6	17	11.6	69.9	

CONCLUSION

From present case series and previous literature we can interpret that REP can be undertaken in mature nonvital permanent tooth with apical pathology using PRP/BC as scaffold, and thus can be used as an alternative to RCT.

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