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Evaluation of Regenerative Endodontic Procedure in Human Permanent Teeth with Persistent Periapical Pathology after Conventional Root Canal Treatment: A CBCT Study

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Abstract
Background: *Regenerative Endodontic Procedure (REP) is a 'paradigm shift' in the treatment of non-vital immature permanent teeth and being used recently in non-vital mature permanent teeth also. However, it's use is yet to be evaluated for management of RCT failure cases with apical periodontitis.*
Aim: *Evaluation of REP in failed RCT cases with persistent periapical pathology using Blood Clot, PRF and PRP as scaffolds.*
Method: *Teeth were randomly divided into control i.e Re-RCT group (n=10) and three experimental groups with blood clot (n=11), PRF (n=13) and PRP (n=9) as scaffolds. After properly removing the gutta percha, cleaning, shaping and irrigation, obturation was done in control group and in experimental groups, REP was performed with respective scaffolds as per the protocol of AAE and ESE. Clinical and radiological evaluations were done after every 3 months through IOPAR and quantitatively using CBCT scans.*
Statistical analysis: *Pre and post operative data was analysed using IBM SPSS Statistics for Windows, Version 26.0.*
Results: *Significant reduction in periapical lesion size and gain in bone density was seen both in control and experimental groups from the baseline to the last follow up period at which time however, this reduction and gain among the 4 groups were not significant.*
Conclusion: *Re-RCT procedure and REP using Blood clot, PRF, PRP as scaffolds are equally effective in resolution of clinical signs & symptoms and bony healing in failed RCT cases with persistent apical periodontitis therefore REP may be considered as one of the treatment option in such cases.*
Keywords: *- REP, Blood clot, PRF, PRP, AAE, ESE, CBCT scan*

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I. Introduction

Regenerative endodontic procedures (REPs) can be defined as biologically based procedures designed to physiologically replace damaged tooth structures, including dentin and root structures, as well as the pulp dentin complex[1].Successful REP results in elimination of clinical signs and symptoms, resolution of apical periodontitis, thickening of the canal walls and/continued root development with or without apical closure. Since REPs restore the vitality of the tooth, it restores the immune defense mechanisms to protect the tooth from foreign invaders[2,3]. It is a 'paradigm shift' in the treatment of non-vital immature teeth. Also, it is being used to successfully treat human mature permanent teeth with necrotic pulps[4]. However, reports on using REPs in endodontically treated permanent teeth with persistent periapical pathology are very limited[5,6]

Therefore, the present clinical study was aimed to evaluate REP in such clinical situation using Blood Clot, Platelet Rich Fibrin (PRF) and Platelet Rich Plasma (PRP) as scaffolds.

II. Materials And Methods

Total number of 60 teeth in 54 patients aged between 15-50 years were selected after taking thorough medical and dental history. All findings of clinical and radiological examination- both IOPAR & CBCT in axial, sagittal and coronal views (Fig:-1A & 1B) and pulp sensibility test were recorded. The inclusion criteria of the cases were- i) Endodontically treated teeth with persistent periapical radiolucency ii) Non-allergic to medicaments and antibiotics necessary to complete procedure (American society of Anesthesiology -ASA1 or

ASA 2). iii) No signs of - root fracture, active resorption, pathologic mobility or probing depth > 3mm. iv) Systemically healthy patients agreed to sign the informed consent form. 4 patients with 6 teeth were excluded as they declined to participate at the end. The research work was done for dissertation for post graduate study of the Institute under the guidance of same postgraduate teacher of the department. Details of proposed treatment plans were explained to the patients & parents and written consent was taken. The study protocol was approved from Institutional Ethics Committee.

First appointment was conducted on 54 teeth in 50 patients. Local anesthesia was achieved using 2% lidocaine with 1:100000 epinephrine. Rubber dam(Coltene Whaledent, Germany) was placed. Root canal filling material i.e GP was accessed and removed using H-file (Fig:- 1C), without using any solvent. The working length was determined using apex locator (Dentsply Propex Pixi) and was confirmed by IOPAR (Fig:- 1D). Minimal instrumentation was done accompanied by copious irrigation with 20ml of 1.5% sodium hypochlorite solution for 5 minutes. Then, the canal was dried with paper points and calcium hydroxide (Ultracal XS of Ultradent, USA) was placed inside the canal (Fig:- 1E)and the access cavity was sealed with Cavit (3MESPE,Germany).Total 48pts (52 teeth)attended the first visit and were recalled after 3-4 weeks.

41 subjects (44 teeth) responded to second appointment and were re-assessed for any signs and symptoms; Ca(OH)2 was flushed away from the teeth through copious & gentle irrigation with 20ml of 17% EDTA (Desmear, Ahmedabad, India) for five minutes which was followed by saline irrigation. Then the canal was dried with paper points and were allocated randomly to different groups.

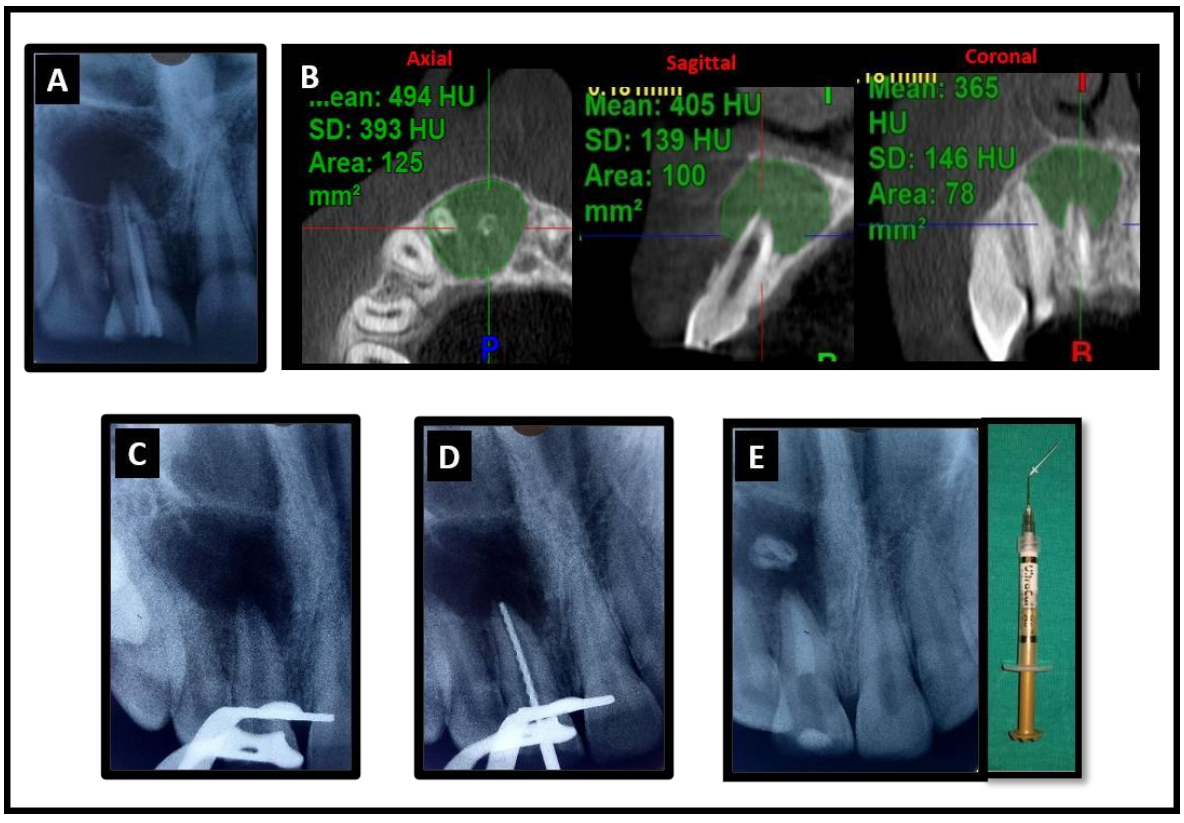


FIGURE 1:- A. Pre-op IOPAR showing poorly obturated 11 along with periapical pathology **B.** Pre- op CBCT-Axial, Sagittal & Coronal view **C.**GP removed **D.**WL estimation **E.** Calcium hydroxide placed inside the canal (erroneously some amount has been pushed beyond apex)

Therefore, in case of **Control group (Re-RCT)**, the canal was obturated using Gutta Percha (Fig:- 2A & 2B) followed by composite restoration (Ivoclar Vivadent, Switzerland) (Fig:- 2C & 2D)**.**

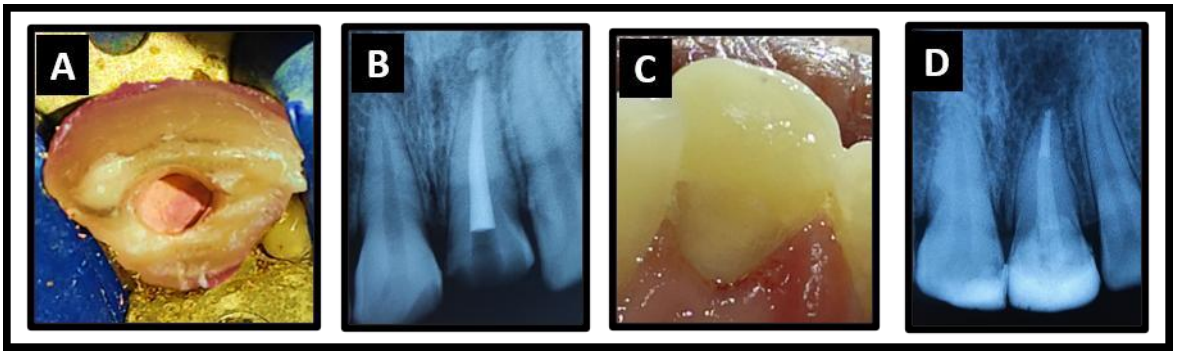


FIGURE 2:- A&B. Canal obturated with GP. **C&D.** LC composite build up done

In the **Blood Clot group**, a size #20 K-file was rotated 2mm beyond the apical foramen under local anesthesia without vasoconstrictor to induce bleeding within the canal (Fig:- 3A). After waiting for 10 minutes to form a blood clot (Fig:- 3B), collagen plug **(**CollaCote; Integra Life Sciences**)** was placed over the blood clot (Fig:- 3C) to facilitate packing of 3mm of MTA over it upto CEJ (Fig:- 3D). Checking it's setting, coronal restoration was done using the same composite resin. (Fig:- 3E & 3F)

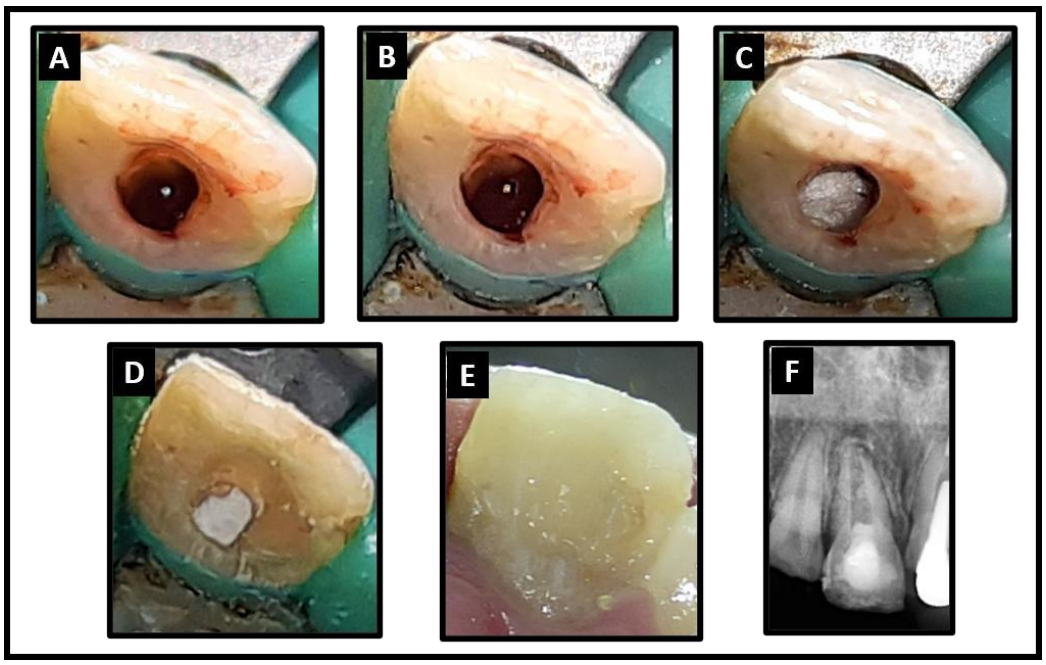


FIGURE 3:- A. Bleeding induced in the canal **B.** Blood clot formed**. C.** Collacote placed over the blood clot. **D.** 3mm of MTA packed over the Collacote. **E.** Composite restoration done. **F.** Immediate Post op IOPAR

In the **PRF group**, the protocol suggested by Dohan et al was followed to prepare the PRF. 10ml of blood was collected (Fig:- 4A) and transferred to a test tube (Fig:- 4B), centrifuged (Remi R8C, Remi Instruments, Mumbai, India) at 400 x g (2114 rpm) for 10 min (Fig:- 4C). The middle layer of PRF (Fig:- 4D) was taken out using sterile tweezer (Fig:- 4E) leaving the top and bottom layers of plasma and RBC respectively and squeezed between gauge pieces (Fig:- 4F) and autologous fibrin membrane thus obtained was then pushed within the canal with help of hand plugger (Fig:- 4G). 3mm of MTA (Fig:- 4H & 4 I) was placed over PRF followed by placement of coronal restoration with the composite resin. (Fig:- 4J & 4K)

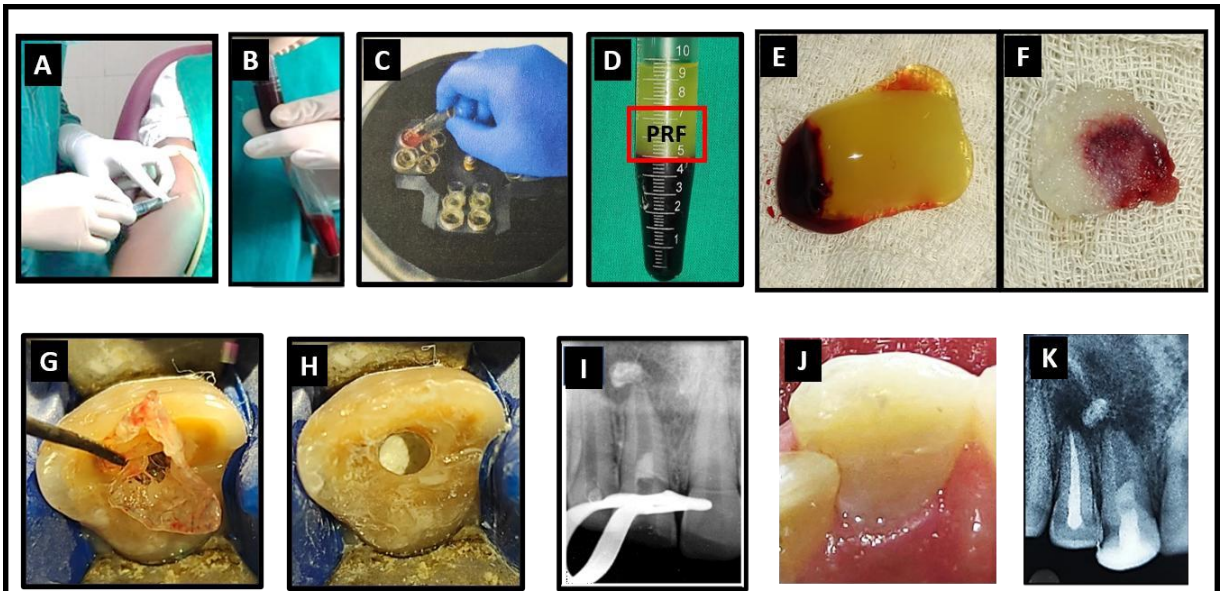


FIGURE 4:- A. Blood being drawn from antecubital vein. **B&C.** Blood transferred to a test-tube to be placed in the Remi R 8C centrifugation machine**. D.** PRF obtained (middle layer). **E.** PRF separated from platelet poor plasma and RBC **F.** Compressed under sterile gauze. **G.** PRF inserted into the canal. **H&I.** 3mm of MTA placed over PRF below CEJ **J.** Restored with composite resin **K.** Immediate post-op IOPA showing REP in 11 and RCT in 12.

For **PRP group**, 5ml of blood was collected and transferred to a glass test tube containing anticoagulant 3.8% sodium citrate (Universal chemicals, Kolkata, India), centrifuged (Remi R8C, Remi Instruments, Mumbai, India) at 300 x g (1831 rpm) for 5 minutes. The separated plasma with buffy coat (Fig:- 5A) was transferred to other glass test tube (Fig:- 5B) discarding the layer RBCs and subjected to second centrifugation at 700 x g (2797 rpm) for 17 minutes. The second spin separated PRP at the bottom (lower 1/3rd) and clear straw colored serum Platelet Poor Plasma (PPP) at the top (upper 2/3rd) (Fig:- 5C). Outermost layer of PPP was removed with syringe and discarded and the remaining PRP (Fig:- 5D) is mixed with 10% calcium chloride and shaken well (Fig:- 5E). The freshly prepared PRP was then taken in an insulin syringe and injected into the canal below the level of CEJ and allowed to clot for 10 min. (Fig:- 5F)**.** Then Collacote placed over PRP (Fig:- 5G) and MTA (Angelus)of 3mm thickness was packed over it till the level below the CEJ (Fig:-5H). On verifying its setting after 15-20 min, composite resin restoration was done (Fig:- 5 I & 5J) similarly.

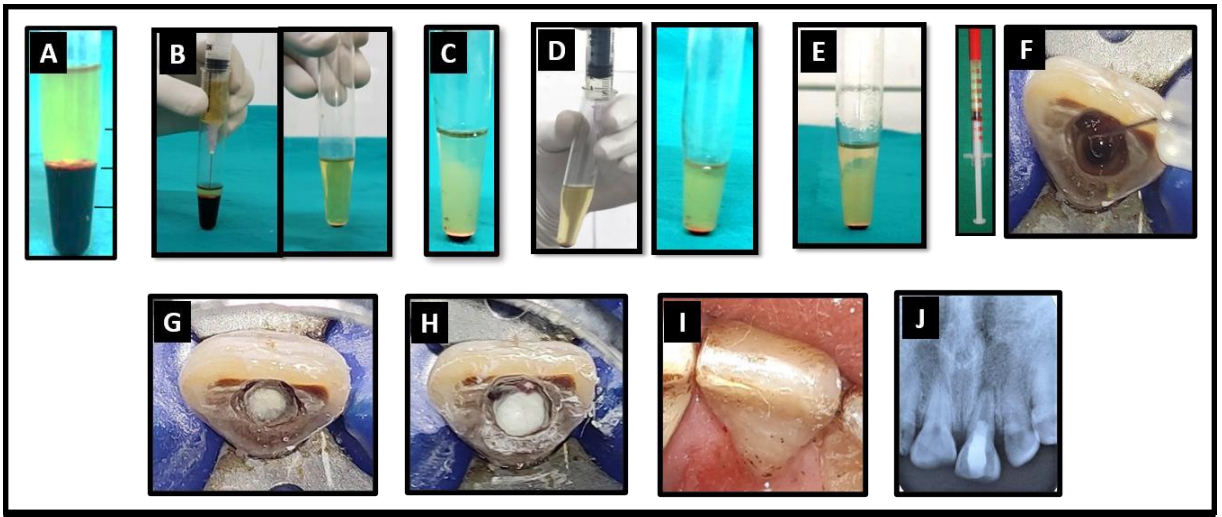
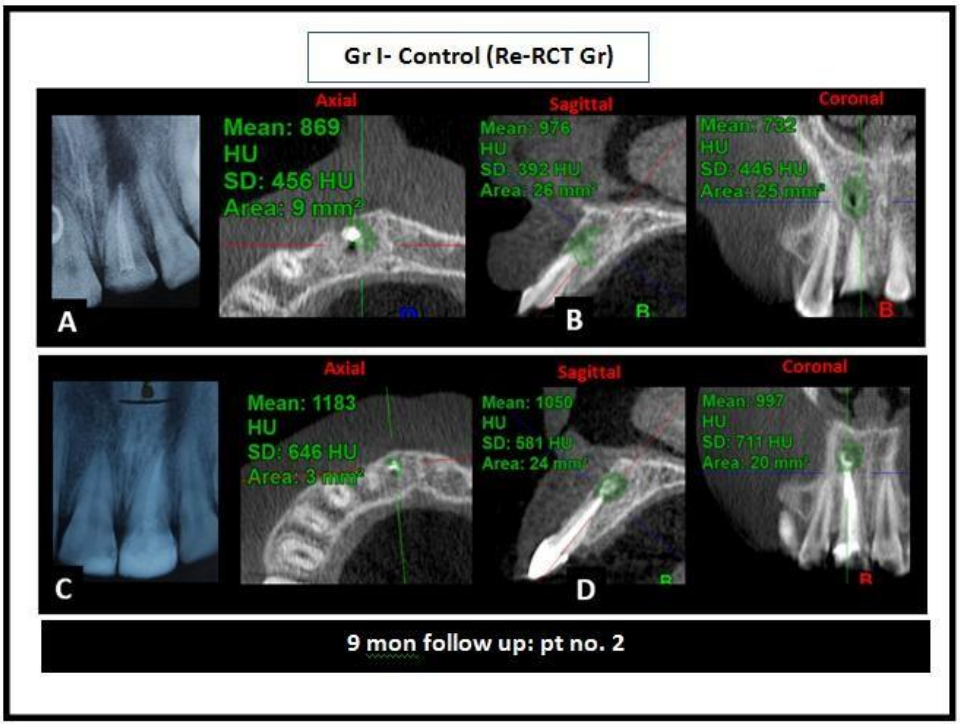
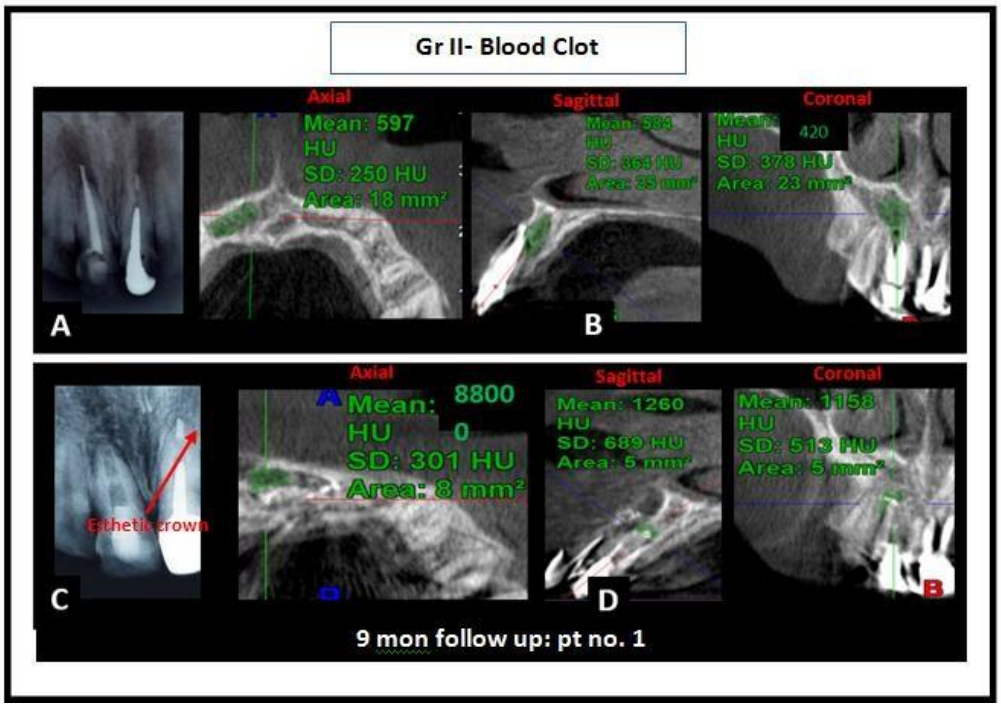


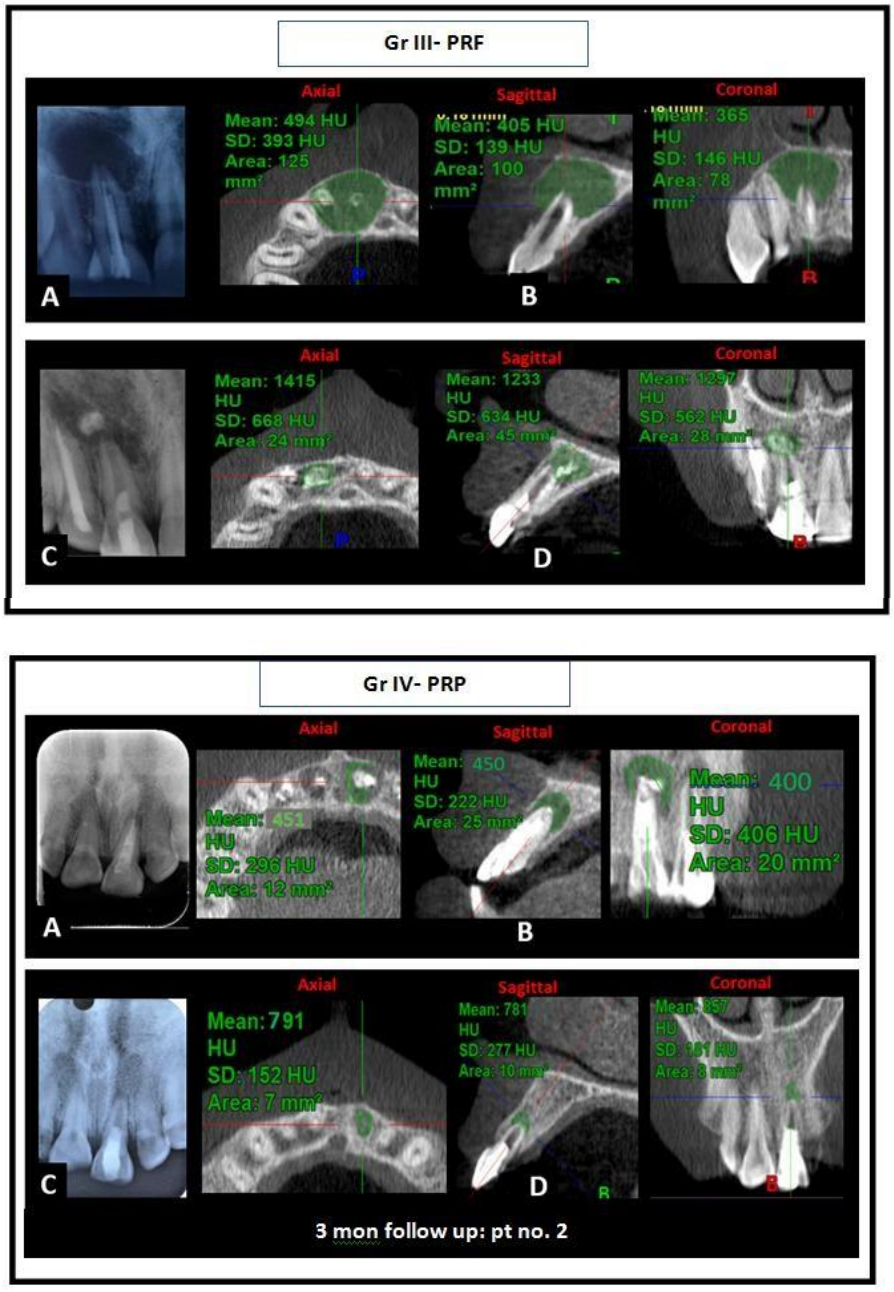
FIGURE 5:- A. RBC separated from plasma and buffy coat. **B.** Plasma and buffy coat taken into another test-tube **C.** Freshly prepared PRP in the middle with PPP at top. **D.** PRP after removal of PPP **E.** PRP activated by addition of 10% calcium chloride. **F.** Freshly prepared PRP was taken into the insulin syringe and inserted into the canal. **G.** Collacote placed over PRP clot. **H.** 3mm of MTA placed over Collacote **I.** Restored with composite resin **J.** Immediate post-op.

The patients were recalled every three months. The teeth were assessed clinically and radiographically both through IOPAR and CBCT scans- axial, sagittal and coronal views to evaluate success of REP. Vitality test with cold (Endofrost, Coltene Whaledent, Langenau, Germany) and electric pulp test (EPT) (Confident Dental, Bangalore, India) was done.

Radiological healing assessed through IOPAR was quantitatively evaluated through CBCT scans and only the data at the last follow up visit of each case was tabulated for statistical analysis. However, pulp vitality could not be found positive in any of the cases under the study.







Gr I, II, III and IV: A. Pre-op IOPA-R. **B.** Pre-op CBCT:- Axial; Sagittal and Coronal view. **C.** Last follow up IOPA-R. **D.** Last follow up CBCT:- Axial; Sagittal and Coronal view.

STATISTICAL ANALYSIS

The collected data was tabulated in a spreadsheet using Microsoft Excel 2019 and then statistical analysis was carried out using IBM SPSS Statistics for Windows, Version 26.0. (Armonk, NY: IBM Corp). Descriptive statistics were used to report the values of central tendency(median) and measures of dispersion (inter-quartile range). Chi-square test of proportions was employed to evaluate the demographic and categorical variables. Non-Parametric tests were carried out for inferential statistics. Wilcoxon Signed Rank Test was carried out to compare the pre-op and follow up data for the four groups individually and the Kruskal-Wallis Test was carried out to compare the mean difference in ranks (preop-last follow up) between the four groups for the given study variables (reduction in lesion size and increase in bone density units) including age. The P-value of 0.05 was considered as the level of significance.

III. Results

Thus, **final outcome evaluation was done on 44 teeth in 41 patients**- 10 teeth (10 patients) in control (Re-RCT group) (Chart I), 12 teeth (11 patients) in Blood Clot group (Chart II), 13 teeth (11 patients) in PRF group (Chart III), and 9 teeth (9 patients) in PRP group. (Chart IV)

All teeth were clinically evaluated. There was no pain, tenderness, swelling, discharging sinus or mobility.

The percentage of females in the study population (53.7%; n=22) was greater than the male population(46.3%; n =19). However, a corrected Chi Square ($\chi 2$) test of independence by Monte-Carlo method revealed no significant association. **($\chi 2$ =2.67,P=0.5)**, implying the groups were matched for the gender variable

Also these four groups were matched for their ages [**H(3)= 1.61,P=0.66**)] **(Table I)** and last follow up period**[H(3)= 2.1, P=0.55)] (Table II).**

All the teeth were evaluated through CBCT except 3 teeth in Gr I, 4 teeth in Gr III and 1 tooth in Gr IV. Those were evaluated through IOPAR only as the institutional CBCT unit went out of order and the patient did not turn up later.

The Median of periapical lesion size (mm^2) at last follow up visits was significantly higher [**Control** (Z= 5.9, P=0.02); **Blood clot** (Z= 3, P=0.008); **PRF** (Z= -2.37, P=0.02) and **PRP** (Z= -2.5, P=0.01)] than the pre-operative Median of periapical lesion with the average percentage gain of **23.72%, 54.54%, 51.81% and 37.74%** for Control, Blood clot, PRF and PRP group respectively. (Charts I, II, III, IV & Table III)

The Median of bone density (HU) at last follow up visits was significantly higher [**Control** (Z= 2.37, P=0.02); **Blood clot** (Z= 2.5, P=0.01); **PRF** (Z= 5.9, P=0.02) and **PRP** (Z= 2.5, P=0.01)] than the pre-operative Median of bone density with the average percentage gain of **32.07%, 35.42%, 36.86% and 49.10%** for Control, Blood clot, PRF and PRP group respectively. (Charts I, II, III, IV & Table IV)

However, Kruskal-Wallis test provided **very weak evidence** of a difference between the mean ranks of **lesion size reduction** [H(3)= 2.38, P=0.5)] and **bone density gain** [H(3)= 3.64, P=0.3)] **between the four groups**. (Table V & VI).

Radiological bony healing evaluated through IOPAR was expressed by scoring system (0-none, 1- Fair, 2-Good, 3- Excellent). Bony healing was quite evident as most of the teeth were scored Good (Score- 2). (Chart V)

CHART- I

Group I: CONTROL GROUP (RE-RCT)

Pt. No/ Tooth No	Age/ Sex	Last follow up (M)	Preop				Last Follow up				% Red	Preop				Last Follow up				% Gain
			Axial	Sagittal	Coronal	Av.	Axial	Sagittal	Coronal	Av.		Axial	Sagittal	Coronal	Av.	Axial	Sagittal	Coronal	Av.	
			PERIAPICAL LESION SIZE (mm^2)									GRAY VALUE (HOUNSFIELD UNIT)								
1.11	25/M	6	6	5	6	**5.66**	2	3	3	**2.66**	**53**	557	204	529	**430**	936	777	1147	**953.3**	**121.6**
2.11	16/F	9	9	26	25	**20**	3	24	20	**15.66**	**21.7**	869	976	732	**859**	1183	1050	997	**1076.6**	**25.33**
3.21	30/M	6	6	7	4	**5.66**	3	5	4	**4**	**29.3**	918	1096	1008	**1007.3**	1120	1220	1177	**1172.3**	**16.3**
4.11	17/M	6	12	18	17	**15.66**	12	14	17	**14.33**	**8.49**	746	667	738	**717**	789	719	772	**760**	**6**
5.21	17/M	6	34	30	29	**31**	29	26	24	**26.33**	**15.06**	719	633	757	**703**	890	752	845	**829**	**17.9**
6.46	17/M	6	12	25	14	**17**	10	18	15	**14.33**	**15.7**	328	437	166	**310.33**	390	604	550	**514.66**	**65.84**
7.26	25/F	9	26	21	18	**21.66**	12	13	10	**11.66**	**46.16**	615	461	609	**561.6**	825	568	868	**753.6**	**34.18**
8.46	20/F	3	Assessed by IOPAR (Ref. Chart V)																	
9.11	45/M	9																		
10.31	30/F	6																		
MEDIAN	23	**6**				**17**				**14**	Overall % Red.				**703**				**829**	Overall % gain
IQR	**17-30**	**6-9**				**5.7-22**				**4-16**	**23.72**				**430-859**				**754-1077**	**32.07**

Group II: BLOOD CLOT GROUP

Pt. No/ Tooth No		Age/ Sex	Last follow up (M)	Preop				Last Follow up				% Red	Preop				Last Follow up				% Gain
				Axial	Sagittal	Coronal	Av.	Axial	Sagittal	Coronal	Av.		Axial	Sagittal	Coronal	Av.	Axial	Sagittal	Coronal	Av.	
				PERIAPICAL LESION SIZE (mm^2)									GRAY VALUE (HOUNSFIELD UNIT)								
1.	11	15/F	9	18	25	23	**22**	8	5	5	**6**	**72.7**	597	584	420	**533.6**	880	1260	1158	**1099.3**	**106**
2.	11	25/M	9	24	19	12	**18.3**	1	2	1	**1.33**	**92.7**	671	746	823	**746.6**	916	827	1026	**923**	**23.6**
3.	11	45/F	9	3	17	26	**15.3**	1	2	3	**2**	**86.9**	508	696	1158	**787.3**	1476	729	1278	**1161**	**47.4**
4.	21	17/M	12	0	2	2	**1.3**	1	0	2	**2**	**0**	1183	1240	1032	**1151.6**	1619	1470	1420	**1503**	**30.5**
5.	21	27/M	12	4	3	4	**3.6**	1	2	4	**2.3**	**36.3**	577	700	600	**625.6**	841	707	642	**730**	**16.6**
6.	11	28/M	9	73	38	101	**70.6**	28	23	41	**31**	**56**	1077	767	1034	**934.6**	1067	1144	1159	**1059**	**13.31**
	12								25					619				760			
7.	21	50/F	3	31	30	14	**25**	25	15	13	**17.6**	**29.6**	941	623	470	**678**	1144	890	574	**869.3**	**28.21**
8.	21	22/F	9	16	37	20	**24.3**	4	15	9	**9.3**	**61.7**	862	819	863	**848**	1538	1020	1273	**1277**	**50.5**
9.	41	30/F	6	12	11	11	**11.33**	13	11	11	**11.6**	**-2.38**	720	726	703	**716.3**	565	509	532	**535.3**	**-25.2**
10.	12	30/M	6	7	7	22	**12**	7	5	16	**9.3**	**22.25**	908	952	1089	**983**	935	1328	1196	**1153**	**17.2**
11.	21	30/M	6	2	7	3	**4**	0	3	3	**2**	**50**	570	872	1188	**876.6**	2154	1476	1520	**1716.6**	**95.8**
MEDIAN		28	9				**15**				**6**	Overall % Red.				**787**				**1099**	Overall % gain
IQR		22-30	6-9				4-24				2-12	**54.54**				678-935				867-1277	**35.42**

CHART- III

Group III: PRF GROUP

Pt. No/ Tooth No		Age/ Sex	Last follow up (M)	Preop				Last Follow up				% Red	Preop				Last Follow up				% Gain
				Axial	Sagittal	Coronal	Av.	Axial	Sagittal	Coronal	Av.		Axial	Sagittal	Coronal	Av.	Axial	Sagittal	Coronal	Av.	
				PERIAPICAL LESION SIZE (mm^2)									GRAY VALUE (HOUNSFIELD UNIT)								
1.	21	45/F	15	3	17	26	**15.3**	1	2	3	**2**	**86.9**	508	696	1158	**787.3**	1416	729	1278	**1141**	**44.9**
2.	11	17/F	12	125	100	78	**101**	24	45	28	**32.3**	**68.01**	494	405	365	**421.3**	1415	1233	1297	**1315**	**212.1**
3.	21	26/F	9	10	19	25	**18**	8	9	8	**6.83**	**62**	703	503	787	**664.3**	1082	974	1190	**1177.8**	**77.3**
	22							6	6	4							1170	1272	1379		
4.	11	18/F	6	1	7	3	**3.6**	1	4	3	**2.6**	**27.7**	1227	1079	1283	**1196.3**	1431	817	1685	**1311**	**9.58**
5.	13	31/F	9	1	6	3	**3.33**	1	3	1	**1.66**	**50.1**	1346	1334	1489	**1389.6**	1614	1660	1785	**1686.3**	**21.3**
6.	11	35/M	9	59	28	65	**50.66**	49	26	51	**42**	**17.09**	1131	1322	1315	**1256**	1457	1404	1335	**1398.6**	**11.3**
7.	21	25/M	3	55	51	69	**50.33**	25	32	49	**29.33**	**41.7**	833	599	822	**803.8**	872	661	946	**891.83**	**11**
	22				27	45			22	23				955	781			1100	900		
8.	21	19/M	3	Assessed through IOPAR (Ref. Chart V)																	
9.	11	15/F	6																		
10.	11	26/F	3																		
11.	21	21/F	3																		
MEDIAN		25	6				**18**				**6.8**	Overall % Red.				**804**				**1311**	Overall % gain
IQR		18-31	3-9				3.6-51				2-32	**51.81**				664-1256				1141-1399	**36.86**

CHART- IV

Group IV: PRP GROUP

Pt.No/ Tooth No	Age/ Sex	Last follow up (M)	Preop				Last Follow up				% Red	Preop				Last Follow up				% Gain
			Axial	Sagittal	Coronal	Av.	Axial	Sagittal	Coronal	Av.		Axial	Sagittal	Coronal	Av.	Axial	Sagittal	Coronal	Av.	
			PERIAPICAL LESION SIZE (mm^2)									GRAY VALUE (HOUNSFIELD UNIT)								
1. 22	50/F	3	7	11	5	7.66	5	8	4	5.6	**26.8**	861	1204	985	1016.6	915	1456	1221	1197.3	**17.7**
2. 21	29/F	3	12	25	20	19	7	10	8	8.3	**56.3**	451	450	400	433.6	791	781	857	809.6	**86.7**
3. 36	28/M	6	4	15	13	10.6	3	5	9	5.6	**47.1**	888	878	724	830	958	1327	1112	1132.3	**36.4**
4. 11	17/F	9	2	5	5	4	1	3	1	1.66	**58.5**	981	789	836	868.6	1577	1350	1159	1362	**56.8**
5. 46	21/F	9	5	5	5	5	4	4	2	3.33	**33.4**	502	605	445	517.3	762	971	1330	1021	**97.37**
6. 22	17/M	9	20	17	21	19.3	3	10	8	7	**63.78**	786	853	998	879	1439	1204	1543	1395.3	**58.7**
7. 12	17/M	9	2	9	9	6.66	1	7	7	5	**24.9**	678	1202	1178	1019.3	1738	1414	1364	1505.3	**47.6**
8. 12	35/M	9	32	31	65	42.6	24	31	50	35	**17.9**	1301	957	1315	1191	1323	1234	1604	1387	**16.45**
9. 47	28/F	3	Assessed by IOPAR (Ref. Chart V)																	
MEDIAN	28	9				**9.1**				**5.6**	Overall % Red.				**856**				**1280**	Overall % gain
IQR	17-32	3-9				5.4-19				3.7-8	**37.74**				595-982				1049-1393	**49.10**

CHART- V

IOPAR ASSESSMENT				
With periapical pathology (N=8)				
Patient No.	**Tooth No.**	**Age/Sex**	**Last follow up (month)**	**Observation (Bony Healing of lesion upto last follow up) ***
CONTROL (n=3)				
8.	46	20/F	3	**1**
9.	11	45/M	9	**2**
10.	31	30/F	6	**2**
PRF (n=4)				
8.	21	19/M	3	**2**
9.	11	15/F	6	**2**
10.	11	26/F	3	**2**
11.	21	21/F	3	**2**
PRP (n=1)				
9.	47	28/F	3	**1**

**** Expressed in score (0-none, 1- Fair, 2- Good, 3- Excellent)***

TABLE: I-Comparison of age distribution of study subjects between the four groups

Groups	Number (n)	Median	IQR	H value	P value
Group I:Control	10	23	17-30	1.61	0.66
Group II:Blood Clot	11	28	22-30		
Group III:PRF	11	25	18-31		
Group IV:PRP	9	28	17-32		

TABLE: II-Comparison of the last follow-up periods between the four groups

Groups	Number (n)	Median	IQR	H value	P value
Group I: Control	10	6	6-9	2.1	0.55
Group II: Blood Clot	11	9	6-9		
Group III: PRF	11	6	3-9		
Group IV: PRP	9	9	3-9		

TABLE: III- Comparison of the PA Lesion size(mm^2) between the baseline and the follow-up measurements for each group

Descriptive statistics	Preop	Last follow up	Z value	P value
Group I: Control (n=7)				
Median	17	14	5.9	0.02
IQR	5.7-22	4-16		
Group II: Blood Clot (n=11)				
Median	15	6	3	0.008
IQR	4-24	2-12		
Group III: PRF (n=7)				
Median	18	6.8	-2.37	0.02
IQR	3.6-51	2-32		
Group IV: PRP (n=8)				
Median	9.1	5.6	-2.5	0.01
IQR	5.4-19	3.7-8		

n: number of valid analyzed cases

TABLE: IV- Comparison of the bone density units (Δ HU) between the baseline and the follow-up measurements for each group

Descriptive statistics	Preop	Last follow up	Z value	P value
Group I: Control (n=7)				
Median	703	829	2.37	0.02
IQR	430-859	754-1077		
Group II: Blood Clot (n=11)				
Median	787	1099	2.5	0.01
IQR	678-935	869-1277		
Group III: PRF (n=7)				
Median	804	1311	5.9	0.02
IQR	664-1256	1141-1399		
Group IV: PRP (n=8)				
Median	856	1280	2.5	0.01
IQR	595-982	1049-1393		

TABLE: V- Comparison of the difference of radiolucent area of lesions (Δ mm2) of the four groups at last follow-up period with respect to the pre-operative area

Groups	Number (n)	Median	IQR	H value	P value
Group I:Control	7	3	1.7-4.7	2.38	0.5
Group II:Blood Clot	11	7.4	1.3-16		
Group III:PRF	7	11	1.7-21		
Group IV:PRP	8	3.7	1.8-9.9		

TABLE: VI- Comparison of the difference of bone density of the lesions (Δ HU) of the four groups at last follow-up period with respect to the pre-operative area

Groups	Number (n)	Median	IQR	H value	P value
Group I:Control	7	192	126-218	3.64	0.3
Group II:Blood Clot	11	191	124-429		
Group III:PRF	7	297	115-514		
Group IV:PRP	8	435	513-223		

IV. Discussion

Apical periodontitis after root canal therapy is primarily caused by persistent root canal infection or rootcanal reinfection[7,8]. Microbial flora in teeth undergoing secondary root canal treatment is single species of the predominantly gram-positive organism Enterococcus faecalis[9], which is resistant to almost all intracanal medicaments including calcium hydroxide and irrigation systems. But it might not be resistant to immune defense mechanisms of regenerated vital tissue after REP[10].

In the present study, Re-Root Canal Therapy and Regenerative Endodontic Procedure mediated by BC, PRF and PRP was performed in endodontically treated teeth with persistent apical periodontitis. Treatment outcome was compared through clinical and radiological evaluation both by IOPAR and quantitatively by CBCT scans. Re-RCT was performed as per time tested protocol. Since no definite guideline till date has been established for REP, recommendation by American Association of Endodontics (AAE), European Society of Endodontics (ESE) and various published literature were followed in regard to its different aspects e.g. protocol of cleaning, irrigation, intra-canal medicaments, application of scaffolds, leak proof seal of access cavity etc.

Maintaining balance between bacterial elimination and stem cell survival through proper irrigation protocol and intracanal medication is one of the key factors to successful REP. To achieve this, 20ml of 1.5% NaOCl, 20ml of 17% EDTA and normal saline was used for 5 minutes. Though Triple Antibiotic Paste (TAP) exhibit wide spectrum of antibacterial effect, the concentration (1-5mg/ml) at which it provides adequate anti-bacterial effect without hampering the stem cell survival is difficult to achieve clinically[11]. On the other hand, calcium-hydroxide though less effective against some intra-canal bacterial species, but it's use at any concentration is associated with lower cytotoxicity to stem cells[12].Moreover, it can be easily removed (approximately 80%) from the canals, unlike TAP of which greater than 80% remains within the canal (>350µ within the dentinal tubules)[13].

In the present study three most commonly used host derived scaffold i.e Blood Clot, PRF and PRP were used and result of REP outcome was compared with the result of control group (Re-RCT) using IOPAR and quantitatively through CBCT scans.

Blood clot as the scaffold, though associated with ease of operation and minimum armamentarium, is difficult to induce bleeding up to the level of CEJ[14] and placement of MTA over the blood clot is also challenging.

PRP produces a very high concentration gradient of platelets whose granules are rich in Platelet Derived Growth Factor (PDGF), Transforming Growth Factor (TGF−β), Epidermal Growth Factor (EGF), and Vascular Endothelial Growth Factor (VEGF), which are important for angiogenesis and improve tissue vascularization[14]. Also, the effect of PRP on bone and dentine regeneration is limited as it releases 81% of total TGF-β1 and PDGF within 1st day with remarkable reduction at 3, 7, and 14 days such that maximum release occurs before actual cell ingrowth[14].

PRF, which was used in this study, releases growth factors from its fibrin mesh of unique nature in a sustained manner with the peak reaching at 14 days[14] which is the time of cell growth. Growth factors important for regeneration like VEGF, TGF- β1, PDGF, EGF, etc which are known to promote cell migration, adhesion, proliferation and differentiation of periapical stem cells are enmeshed in PRF. This results in periapical bone regeneration, root maturation through development of pulp-dentin complex[15].Since, there is no extra chemical agent administration, it is more physiologic and free of any possible adverse reactions[14].

Chemical solvents were not used for removing the previous root canal filling material to avoid associated cytotoxicity. Chloroform and other gutta-percha solvents have been shown to be highly toxic in several ex vivo studies[16].

The MTA that has been used in the present study as a plugging material is easy to handle and sets within the clinically acceptable time period.

During the course of follow up period, all the teeth in Control group i.e. Re-RCT cases and 3 Experimental i.e. REP groups were functional and clinically asymptomatic.

Considering the limitations of 2D imaging, 3D imaging using CBCT for quantitative evaluation was done in this study. CBCT has been recommended as assessment tool in REP by AAE 2018[17]and ESE 2016**.**[18] IOPAR evaluation were generally less reliable than the CBCT measurements[19,20] and the outcome of root canal treatment determined with IOPAR could be untrue.[21] Healing of periapical lesion size after root canal treatment was seen using both IOPAR and CBCT.

Periapical lesion healing was assessed by **reduction in lesion area measurements and gain in bone density using Gray value.** Periapical lesion measurements were done in all the **three planes i.e. axial, sagittal and coronal planes** by tracing the area of bone rarefaction and mean of three was used as a final measurement. On completion of tracing the area, value of bone density (HU) was also displayed in the scan.

Significant reduction in periapical lesion size and increase in bone density was seen both in control and 3 experimental groups.

However, **this difference** in reduction in periapical lesion size and the gain in bone density among the 4 groups at last follow up visit was **not significant.** That means in result of Re-RCT and REP outcome did not vary at the end of average period of follow up of the present study.

Therefore, all the above findings of the present study provide evidence that BC, PRF and PRP mediated REPs have potential to be used for treating the endodontically failure teeth with persistent apical periodontitis instead of conventional Re-RCT procedure. Additionally root canals of REP treated teeth would be filled up by viable tissue with immune system, defence mechanism and proprioception. Studies with histological contribution to REP shows that in most of the cases the vital cementum tissue has grown within the root canals[22,23]**.**

There are number of studies of REP in permanent teeth using BC, PRP and PRF as scaffold and it was found that there was no significant difference in efficacies of these 3 scaffolds.

Ulusoy et al[24] (2019) and Murray et al[25] (2018) using IOPAR and Markandey S & Adhikari HD[26](2021) using both CBCT and IOPAR performed REP using BC, PRF and PRP on permanent teeth**.** In all these studies, no significant difference was found in periapical bone healing among the three study groups i.e. Blood clot, PRP and PRF but these studies did not include comparison of Re-RCT with the REP procedures.

No significant difference in bony healing was observed on comparing **PRF and Blood clot group** by **Prabhakar et al**[27] **(2016)**, **Lv et al**[28] **(2018) using IOPAR** and **Alrashidi et al**[29] **(2021)** using both CBCT and IOPAR in immature permanent teeth. Similar results were found on comparing **BC with PRP** by **Alagl et al**[30] **(2017) using CBCT**, **ElSheshtawy et al**[31]**(2020) using both IOPAR and CBCT** and **Bezgin et al**[32] **(2015)** using IOPAR and on comparing **PRP and PRF by Rizk et al**[33] **(2019), Alrashidi et al**[29] **(2021) using IOPAR and Adhikari HD et al**[13] **(2021)** using IOPAR and CBCT in immature permanent teeth**.**

Conversely, a study by **Narang et al**[34] **(2015)** using IOPAR in immature permanent teeth reported significant periapical healing **(using scores) in PRF group** as compared to BC and PRP group. Whereas, a study by **Shivashankar et al**[35] **(2017)** conducted using IOPAR showed **periapical healing to be statistically significant in PRP group** as compared to BC and PRF group.

In a study conducted by **Rizk et al**[36] **(2019)** using IOPAR in immature permanent teeth **on comparing BC and PRP**, it was reported that **PRP treated teeth showed a statistically significant increase in periapical bone density when compared with BC group**. This study was in contrast to the findings of **Alsharidi et al**[29] **(2021)** in which **BC treated teeth showed a statistically significant bone healing as compared to PRP group using IOPAR**.

There are some case reports which had also documented the success of REP in the failed endodontic cases, both in permanent mature (**Saoud et al**[37] **2015, Turk et al**[38] **2020** and **Sharma S et al**[39] **2021**) and immature teeth (**Orduna et al**[40] **2017, Cymerman et al**[41] **2019, Miltiadous et al**[42] **2015, Nosrat et al**[43] **2021 & Sharma S et al: 2021**[39]). **But search literature failed to provide any study showing comparison of outcome of REP procedures with result of Re-RCT.**

V. Conclusion

Within the parameters & constraints of this study, it may be concluded that **Re-RCT procedure and Regenerative Endodontic Procedure using Blood clot, PRF, PRP as scaffolds are equally effective in resolution of clinical signs & symptoms and bony healing** in human permanent teeth with persistent periapical pathology after conventional root canal treatment and REPs may be a better option in this regard.

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