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Comparative Evaluation of Healing After Periapical Surgery Using Hydroxyapatite Crystals and Platelet Rich Fibrin with and without Guided Tissue Regeneration Membrane:A CBCT Study.

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Abstract:
Aim *– To evaluate and compare the postsurgical bony healing in periapical region in apicoectomy cases using Blood Clot, a mixture of Hydroxyapatite crystals (HA) and autologous Platelet Rich Fibrin (PRF) and Combination of HA and PRF along with Guided Tissue Regeneration (GTR) membrane in the bony cavity.*
Material & Method *– 24 patients who had apical lesion size more than 5mm or through and through lesion and were reluctant to accept an attempt of nonsurgical method, were randomly allocated to the study groups –*
Group I – Blood Clot
Group II – HA + PRF mixture
Group III – HA, PRF along with GTR membrane
After thorough Root canal Treatment, Endodontic microsurgery was performed then the wound was closed with respective filler material within the bony wound. The patients were recalled post operatively for clinical and radiological evaluation. Healing of bony wound was evaluated on the basis of increase in bone density and reduction in radiolucent area with CBCT.
Result*- It was observed that median increase in the bone density and reduction in radiolucent area was significantly different among the three study groups with maximum healing in Group III, followed by Group II and lowest in Group I.*
Conclusion*- PRF and HA combination along with resorbable GTR membrane was seen to be promising in inducing and accelerating hard tissue regeneration.*
Key words*: PRF, HA, GTR, Endodontic microsurgery, Bone density*

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I. Introduction

Regeneration of bone in periapical bone defects in root-end resected tooth following periapical surgery is a major challenge to endodontist, especially in case of larger or through and through lesions. Wound heals either by repair or regeneration depending on its nature; progenitor cells availability; signalling molecules; and micro-environmental signals such as adhesion molecules, extracellular matrix, and associated non-collagenous protein molecules.[1] Regeneration is defined as the reproduction or reconstitution of a lost or injured part of the body in such a way that the architecture and function of the lost or injured tissues are completely restored.[2] Since repair is undesirable, regenerative approaches that aim to reinstate the lost periodontium and the adjoining connective tissue, have been introduced.[3] It frequently includes the use of bone grafting materials and barrier membrane to reassure the growth of these surrounding tissue, while excluding unsolicited cell types such as epithelial cells or fibroblasts.[4]

Porous hydroxyapatite (HA) has been used to fill the periodontal intra-bony defects, which has resulted in clinically satisfactory outcomes.[5] It has been shown that porous HA bone grafts have exceptional osteo-conductive properties, which permit extension of osteogenic cells from existing bone surfaces into the adjacent bone defect.[6]

Platelet-rich fibrin (PRF) is a second-generation platelet concentrate which is a rich source of various growth factors and cytokines embedded within the fibrin clot.[7]

PRF is osteo-inductive, and HA is osteoconductive. This combination offers collaborative effect on bone regeneration. Clinical data reveal that it will be a favorable matrix for excellent healing without excess inflammation[8] because of several advantages.[9]

i. Fibrin clot provides mechanical and biological connector between HA particles
ii. This integration of fibrin net promotes various stages of healing such as cellular migration, especially endothelial cells for the purpose of neoangiogenesis, vascularization and graft survival.
iii. As the fibrin matrix is resorbed in passage of time, growth factors are gradually released and healing process continuously progresses.
iv. Cytokines and incorporated leukocytes play significant role in controlling inflammation within the graft material.[10]

Guided tissue regeneration therapy using GTR membrane or barrier membrane introduced in 1980s has been widely used to regenerate lost periodontium suffering from periodontal disease. Scientific evidences indicate that principles of GTR can be effectively applied in endodontic surgery to correct bony defects restricted to periapical region. The placement of GTR membrane over an osseous defect can prevent the faster proliferating oral epithelium and gingival connective tissue from growing into bone defect, allowing the cells of the periodontal ligament and endosteum to populate and regenerate the lost tissue. [11]

Study of Sculean*et al.*[12] revealed that the combination of barrier membrane and graft materials may result in histological evidence of periodontal regeneration, principally bone repair. Pradeep *et al.*[13] in their study showed that HA and PRF mixture increased the regenerative effects in the treatment of three wall intra-bony defects. But, clinical study in the context of periapical surgery is sparse. Therefore, the purpose of the present study was to evaluate and compare the post-surgical bone regeneration in the periapical region in apicoectomy cases, using Blood Clot only, a mixture of HA&PRF and combination of HA& PRF along with resorbable GTR membraneon the basis of increase in bone density and reduction in radiolucent area at subsequent follow-up periods after performing the surgery.

II.Material and Methods

Twenty-four patients who were systemically healthy, non-smokers; with a periapical lesion in relation to nonvital teeth of anterior region of either arch diagnosed by their clinical signs, symptoms, and IOPA R findings who attended the OPDof Dept. of Conservative Dentistry and Endodontics of the Dental College and Hospital were selected for the study. Those patients had an apical lesions size >5 mm, or through-and-through lesions and were reluctant to accept the attempt of non-surgical method of management.The study protocol was approved by the institutional ethics committee, and informed consent was obtained from all selected patients under the Helsinki Declaration.

The selected patients were scanned by CBCT with SkyView CBCT® Scanner (My-Ray Dental Imaging, Imola, Italy) pre-operatively for baseline measurements on the basis of area of periapical lesion in mm^2 and its bone density expressed inHU. An attempt was made to keep the field of view as narrow as possible (7 cm × 7 cm × 7 cm) to limit the radiation exposure as per the ALARA (as low as reasonably achievable) principle. The scanner was operated at 90 kV and 10 mA with gray levels of 4096 (12 bit). The digital images were then exported from Skyview CBCT Scanner™ and imported into iRYS viewer software.

Then, these patients were equally divided among three groups with patients in each group, depending on how bony crypt to be filled up, namely-**Group I:**natural blood clot, **Group II:** HA+ PRF mixture, **Group III: HA+PRF+GTR.**

Root canal therapy was meticulously performed in all the affected teeth before apical surgery was planned.

During the surgery, an effective infection control procedure was followed. The area was anesthetized using 2% Lignocaine with 1:80,000 adrenaline. A crevicular and two vertical releasing incisions were given and a mucoperiosteal flap was raised revealing apico-marginal defect of the buccal cortical bone along the root surface of respective tooth/tooths. A bony window was made labially with a no. 6 round TC bur along with copious irrigation using sterile saline solution. Apical 3 mm of root/s were removed using tapered fissure bur along with *en-masse* curettage of the apical pathology and integrity of the palatal periosteum was checked. 0° angulation of resected root ends was ensured. The resected tissue was sent for histopathological evaluation. Retrograde cavities of 3 mm depth were prepared with the help of ultrasonic tip and retrograde fillings were done with MTA (Pro Root, Dentsply, Tulsa, USA).

For the patients underGroup I, bony cavities were left to be filled with blood clot and flap was repositioned and sutured (Figure 1).

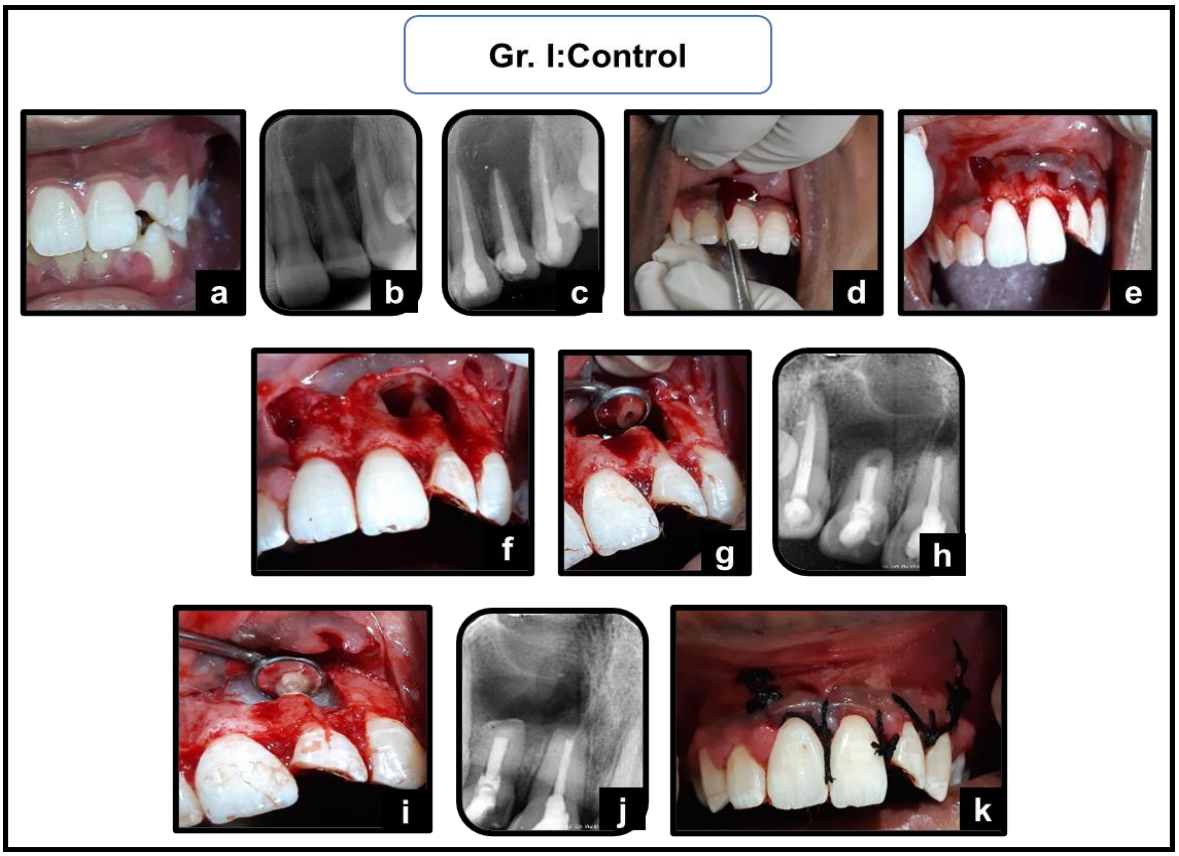


Figure 1: Surgical Procedural Steps for Gr I:Control: Patient No. 1
a)Pre-operative clinical picture **b)**Pre-operative IOPA Radiograph **c)**IOPA Radiograph showing obturation **d)**Incision given e) Flap raised **f)**Osteotomy window prepared g) Apicectomy followed by retrograde cavity preparation done **h)**IOPA Radiograph showing retrograde cavity preparation i) Retrograde cavity restored with MTA **j)**IOPA Radiograph showing retrograde cavity restoration **k)**Flap repositioned and sutures placed

In Group II,PRF was prepared in accordance with the protocol set by Choukroun*et al.*.[14] 10 ml blood was drawn from patient's antecubital vein. It was immediately transferred in a sterile test tube without an anticoagulant and centrifuged at 3000 rpm for 10 mins in a table top centrifuge machine (REMI Model R-8C, India). After centrifugation, PRF was separated from Platelet Poor Plasma at the top and RBC at the bottom of the test tube. PRF was taken out with the help of a sterile tweezer. Bulk of leucocyte layer was removed with the help of sterile scissor and a layer of 1-2mm of it was allowed to remain along with the PRF. It was then mixed with HA bone graft (G-BONE, INDIA) in a sterile dappen dish and the composite graft was then placed into the periapical defect (Figure-2) before suturing.

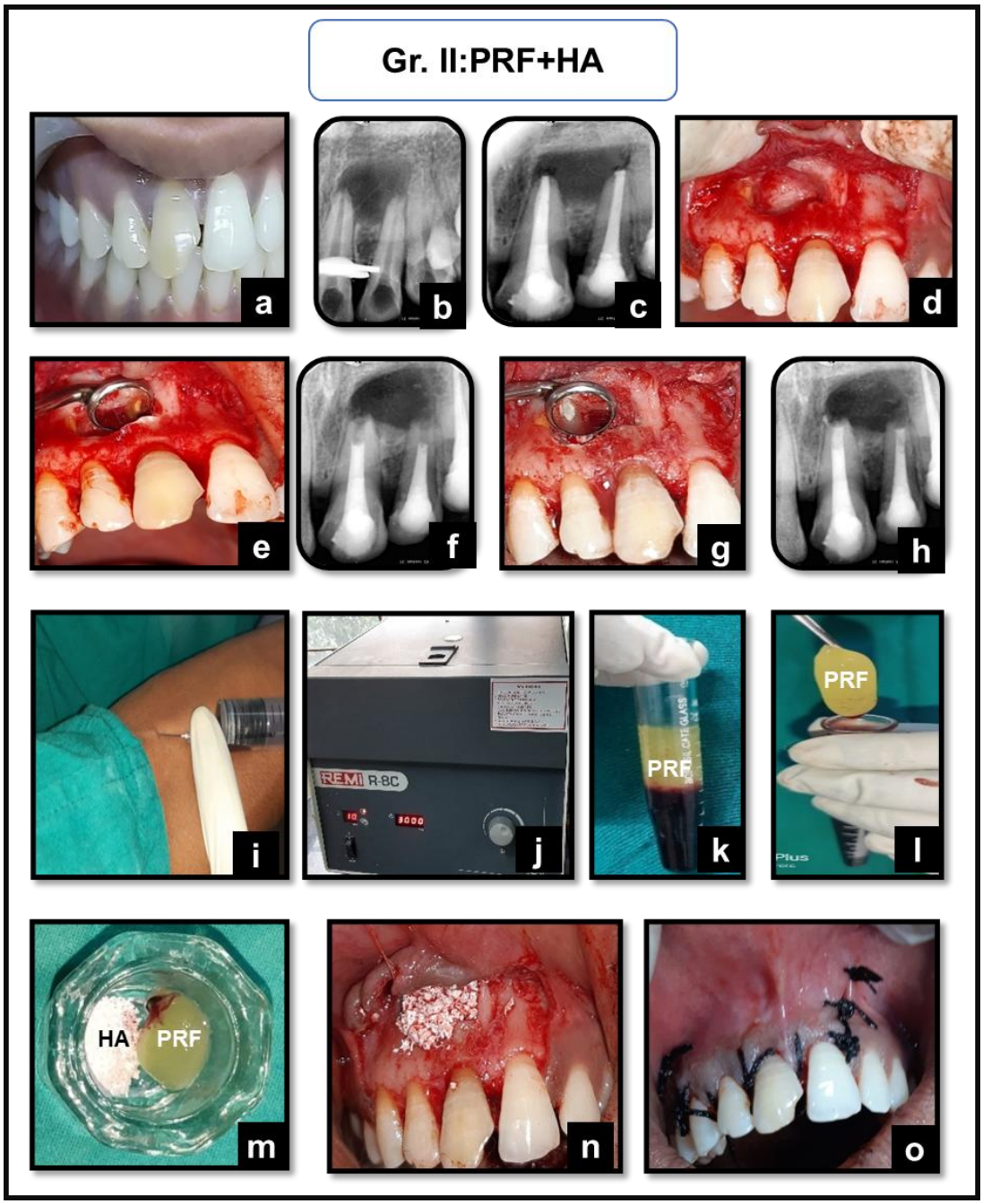


Figure 2: Surgical Procedural Steps for Gr II:PRF+HA: Patient No. 1
a)Pre-operative clinical picture **b)**Pre-operative IOPARadiograph **c)**IOPA Radiograph showing obturation **d)**Incision given and flap raised **e)**Osteotomy window prepared, apicectomy followed by retrograde cavity preparationdone **f)**IOPA Radiograph showing retrograde cavity preparation**g)** Retrograde cavity restored with MTA**h)**IOPA Radiograph showing retrograde cavity restoration **i)**Blood withdrawn from ante-cubital vein of patient to prepare PRF **j)**Blood is centrifuged in the machine(Gforce-400g, Time-10minutes**) k)**PRF in test-tube **l)** Separation of PRF membrane from platelet-poor plasma andRBCs **m)**Mixing of PRF with HA**n)**Bony cavity filled with PRF+HA combination**o)**Flap repositioned and sutures placed

In Group IIIpatients, the defect was filled with mixture of PRF and HA bone graft. GTR membrane (HEALIGUIDE, Advanced Biotech Products (P) Ltd. Tamil Nadu, INDIA) was then placed over it. The size of membrane was trimmed in such a manner that it covered the bony crypt and the apico-marginal defect (were present) 2-3 mm beyond the margin. Amount of the graft material was sufficient enough, so that the membrane did not collapse (Figure 3).

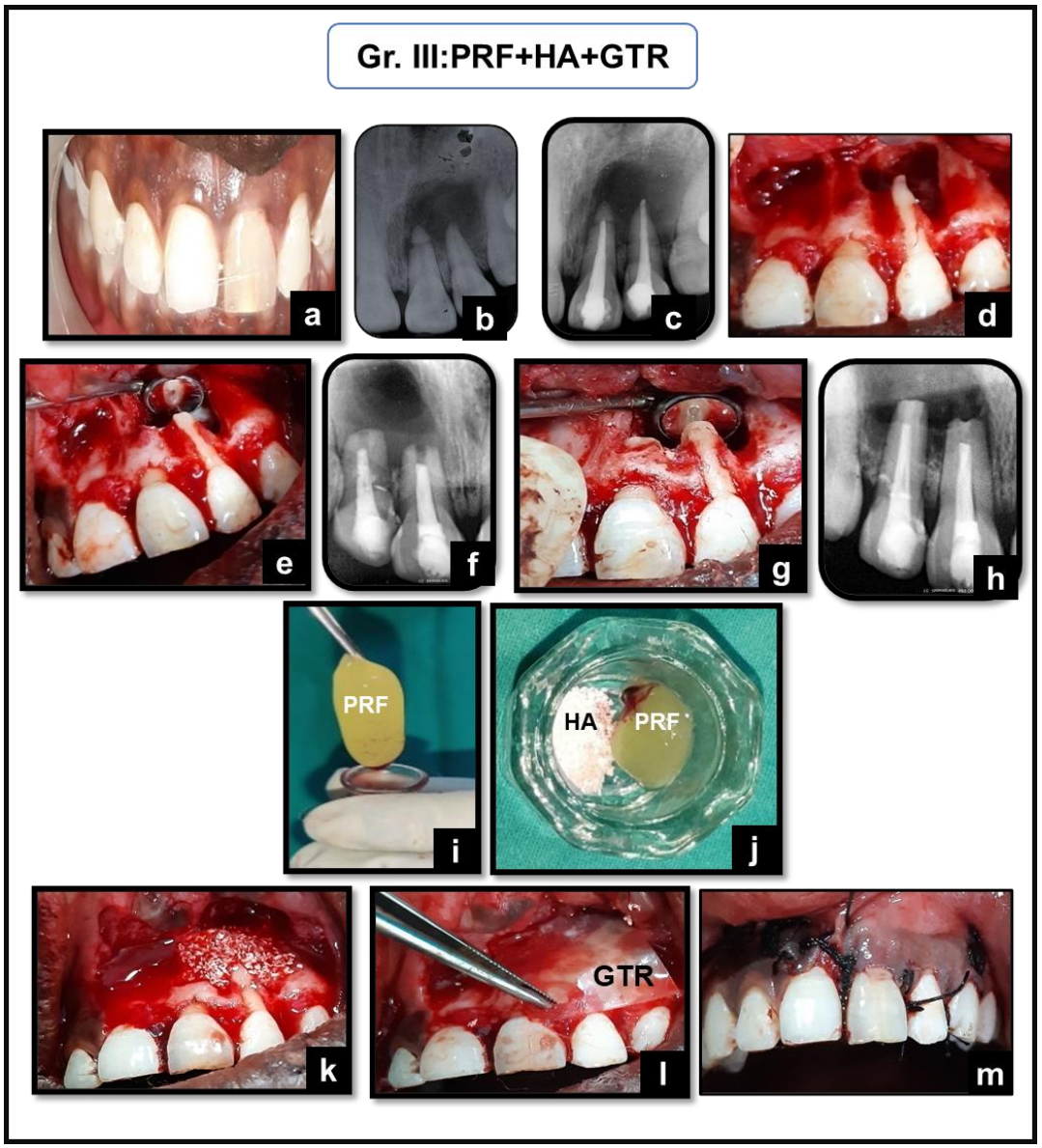
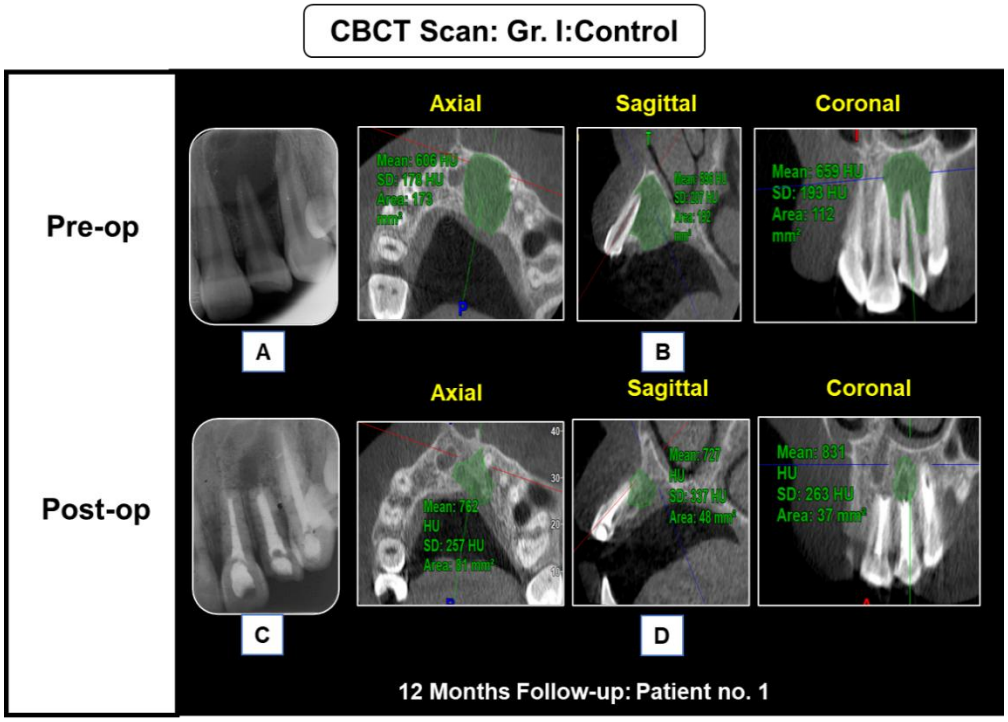
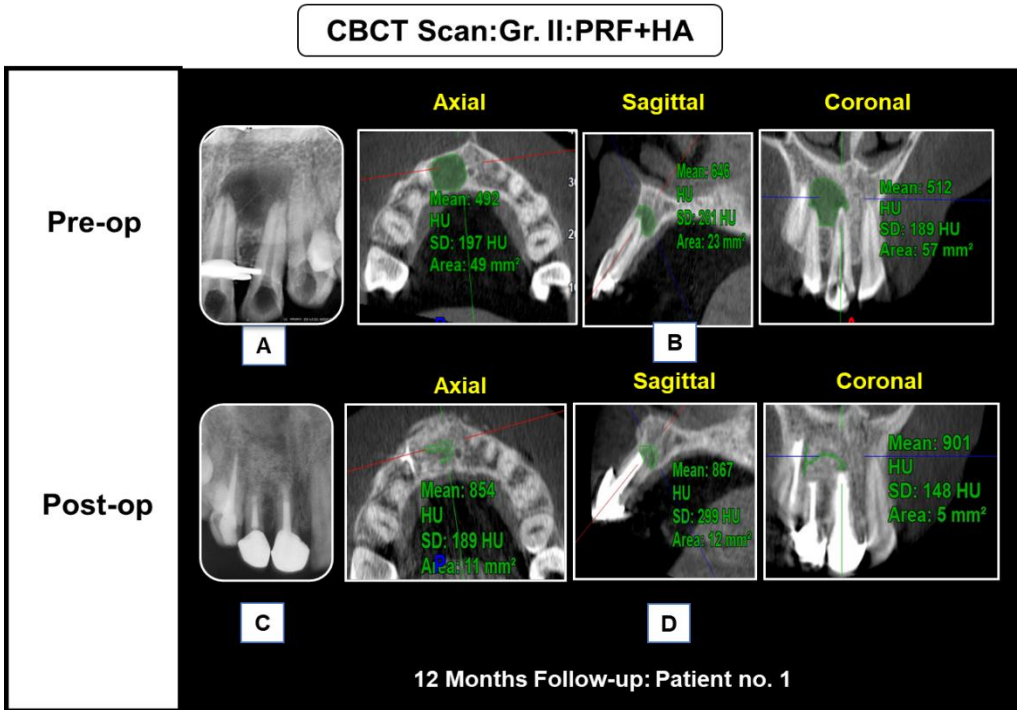


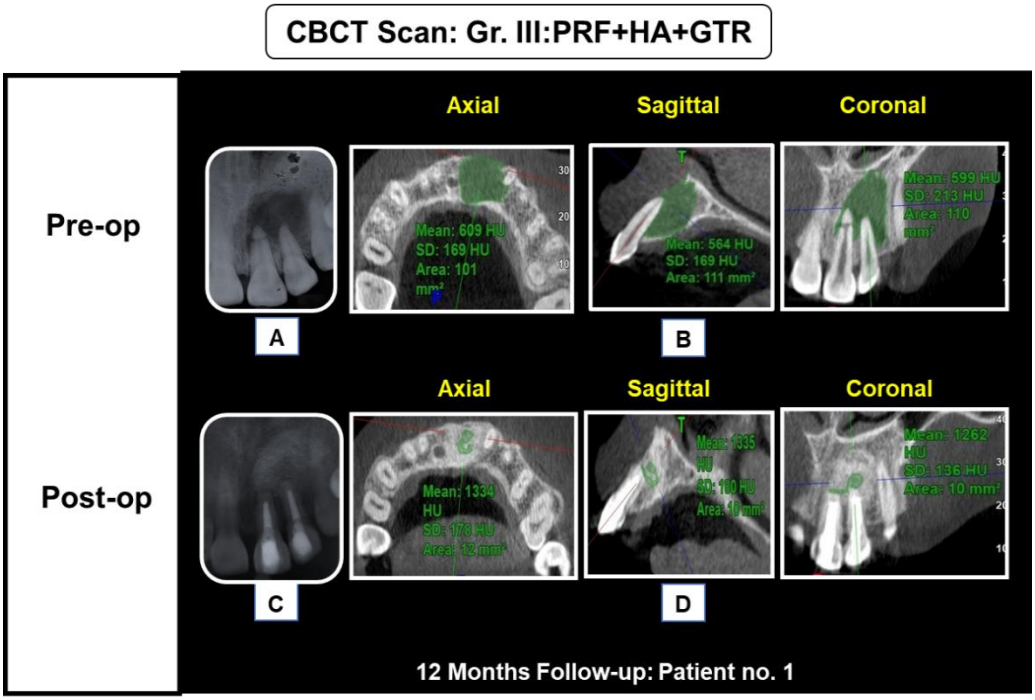
Figure 3: Surgical Procedural Steps for Gr III:PRF+HA+GTR: Patient No. 1
a)Pre-operative clinical picture **b)**Pre-operative IOPA Radiograph **c)**IOPA Radiograph showing obturation **d)**Incision given and flap raised **e)**Osteotomy window prepared, apicectomy followed by retrograde cavity preparation done **f)**IOPA Radiograph showing retrograde cavity preparation **g)**Retrograde cavity restored with MTA **h)**IOPA Radiograph showing retrograde cavity restoration **i)**Separation of PRF from platelet-poor plasma and RBCs **j)**Mixing of PRF with HA **k)**Bony cavity filled with PRF+HA combination **l)**Bony wound covered with the resorbable GTR membrane **m)**Flap repositioned and sutures placed

In all the patients 3-0 silk was used for suturing.Antibiotics and analgesics were prescribed. The patient was disposed with proper advice and asked to report after 7 days for suture removal. After suture removal, the patients were advised for recall visits after 6 &12 months period of time.

On follow-up visits, patientswere evaluated clinically and radiologically both through some of the patients IOPAR & CBCT as were done preoperatively, Due to Corona pandemic of the patients in all 3 groups could not attend last follow-up visit at 12 months as scheduled. The increase in bone density and decrease in area, of the bony defect viewed in CBCT scan from the baseline onwards up to last follow-up (CBCT Scan: Gr I, II, III) visit was evaluated data those obtained were tabulated (Chart I, II, III) and statistical analyzed







Gr I, II and III: A) Pre-operative IOPA Radiograph**, B)**Pre-operative CBCT:-Axial, Sagittal and Coronal sections
C) Last follow-up IOPA Radiograph**, D)**Last follow-up:-Axial, Sagittal and Coronal sections

III. Results

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 three groups individually and the Kruskal-Wallis Test was carried out to compare the mean rank difference(preop-last follow up) between the three groups for the increase in bone density, reduction in lesion size and age. The *P*value of 0.05 was considered as the level of significance.

The present study comprised 54.2% females and 45.8% males; with an age range from 15 to 38 years. The median of the last-follow-up period was found to be 12(6-12) months. The mean ranks of ages and the last follow-up period between the three study groups was not found to be statistically significant(P=0.8 and P=0.52 respectively). Also, no significant association was found in the distribution of patients according to the gender amongst the three study groups(P=0.67). It impliedthat the patients of the three study groups were matched for age, gender and last follow-up periods, thus nullifying the possible effect of these confounding variables on the study outcome.

Within group comparisons revealed a gradual increase in the bone density levels and reduction in lesion size across all time intervals when equated to baseline for all the three study groups (Chart I, II, III). This increase in density and reduction of size was seen statistically significance (P= 0.01)

On comparing the data among the study groups, the following results were obtained:

Regarding the bone density, the median increase in the grey values was significantly different(*P*=0.03) among the three study groups, with maximum increase in Group III [699-(562-909)] followed by Group II [550-(368-606)] and lowest in Group I [288-(194-616)] (Table III,Chart I, II &III).

The median reduction in radiolucent area was also significant different(*P*=0.02) among the three groups but it was highest in Group III [95-(83-151)], followed by Group II [62-(43-81)] and lowest in Group I [59-(40-94)] (Table IV, Chart I, II &III).

Chart No.1: Findings of patients in Group I: blood clot

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)21,22,23	15/M	6	173	192	112	**159**	81	48	37	**55.3**	**65.1**	606	596	659	**620.3**	762	727	831	**773.3**	**24.6**
2)11,12	28/M	12	53	68	78	**66.3**	39	14	38	**30.3**	**54.2**	517	520	485	**507.3**	722	743	601	**688.6**	**35.7**
3)21,22	17/F	6	70	53	63	**62**	42	13	32	**29**	**53.2**	788	689	746	**741**	1056	1043	1019	**1039.3**	**40.2**
4)22,23	19/F	12	236	185	158	**193**	59	25	37	**40.3**	**79**	542	616	774	**644**	891	923	951	**921.6**	**43.1**
5)11,12	22/M	6	80	50	93	**74.3**	28	07	28	**21**	**71.7**	430	452	507	**463**	987	836	972	**931.6**	**101.2**
6)11,12	21/M	12	103	80	114	**99**	34	32	33	**33**	**66.6**	234	342	277	**284.3**	806	1107	935	**949.3**	**233.9**
7)21,22	20/F	6	96	65	118	**93**	36	50	41	**42.3**	**54.4**	543	686	571	**600**	690	1143	661	**831.3**	**38.5**
8)21,22	20/M	12	69	56	84	**69.6**	05	03	04	**04**	**94.2**	559	486	463	**502.6**	1247	1086	1394	**1242.3**	**147.1**
MEDIAN		**9**				**84**				**32**	**Overall % Red**				**554**				**927**	**Overall %Gain**
IQR		**6-12**				**67-144**				**23-42**	**68.73**				**473-638**				**788-1017**	**69.11**

Av.- Average **Red.-** Reduction

Chart No.2: Findings of patients in Group II: HA +PRF

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)11,12,13	20/F	12	49	23	57	**43**	11	12	05	**9.3**	**78.27**	492	646	512	**550**	854	867	901	**874**	**58.9**
2)11,12,13	29/F	12	143	96	102	**113.6**	18	39	28	**28.3**	**75**	291	232	367	**296.6**	1083	1054	1002	**1046.3**	**252.7**
3)11,12,13	38/M	6	86	41	111	**79.3**	28	21	65	**38**	**52.1**	675	812	756	**747.6**	1318	1315	1273	**1302**	**74.1**
4) 11,12	18/F	6	128	64	126	**106**	18	10	09	**12.3**	**88.3**	531	597	600	**576**	1034	1116	1213	**1121**	**94.6**
5) 11,12	17/M	12	69	79	72	**73.3**	03	08	05	**5.3**	**92.7**	622	870	967	**819.6**	1314	1328	1563	**1401.6**	**71**
6) 21,22	16/M	12	90	59	105	**84.6**	25	19	21	**21.6**	**74.4**	594	556	608	**586**	667	604	720	**663.6**	**13.2**
7) 11,12	26/F	6	60	68	92	**73.3**	18	32	22	**24**	**67.2**	631	600	591	**607.3**	1150	1157	1016	**1107.6**	**82.3**
8) 21,22	19/F	6	83	68	112	**87.6**	15	28	35	**26**	**70.3**	477	570	508	**518.3**	997	1214	1187	**1132.6**	**118.5**
MEDIAN		**9**				**82**				**23**	**Overall % Red**				**581**				**1114**	**Overall % Gain**
IQR		**6-12**				**73-101**				**10-28**	**75.06**				**526-713**				**917-1260**	**83.96**

Av.- Average **Red.-** Reduction

Chart No.3: Findings of patients in Group III: HA+ PRF + GTR

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) 21,22	19/F	12	101	111	110	**107.3**	12	10	10	**10.6**	**90**	609	564	599	**590.6**	1334	1335	1262	**1310.3**	**121.8**
2) 11,12	16/M	12	151	116	181	**149.3**	11	22	34	**22.3**	**85**	587	545	628	**586.6**	1053	1645	1095	**1264.3**	**115.5**
3) 21,22	28/M	12	111	70	129	**103.3**	14	20	30	**21.3**	**79.3**	772	717	633	**707.3**	1090	1189	1148	**1142.3**	**61.5**
4) 21,22	34/F	6	139	113	84	**112**	14	18	26	**19.3**	**82.6**	434	367	540	**447**	1024	1019	1177	**1073.3**	**140.1**
5)11,12,13	20/M	12	217	141	193	**183.6**	18	11	27	**18.6**	**89.8**	122	160	160	**147.3**	993	1227	1336	**1185.3**	**704.6**
6) 11,12	22/F	12	131	71	136	**112.6**	26	20	31	**25.6**	**77.2**	558	654	667	**626.3**	1045	1284	1170	**1166.3**	**86.2**
7) 21,22	19/F	6	128	69	125	**107.3**	22	28	31	**27**	**74.8**	482	541	470	**497.6**	1325	1376	1227	**1309.3**	**163.12**
8) 11,12	23/F	12	191	116	222	**176.3**	10	17	25	**17.3**	**90.1**	514	515	573	**534**	1547	1401	1479	**1475.6**	**176.3**
MEDIAN		**12**				**112**				**20**	**Overall % Red**				**560**				**1225**	**Overall %Gain**
IQR		**7.5-12**				**107-170**				**18-25**	**84.6**				**460-617**				**1148-1310**	**139.97**

Av.- Average **Red.-** Reduction

Table I: Comparison of bone density(HU):measured at baseline and last follow-up visit for each group

Descriptive statistics	Preop	Last follow up	Z value	*P* value
Group I: Control	**(n=8)**	**(n=8)**		
Median	554	927	2.52	0.01
IQR	473-638	788-1017		
Group II: PRF+HA	**(n=8)**	**(n=8)**		
Median	581	1114	2.52	0.01
IQR	526-713	917-1260		
Group III: PRF+HA+GTR	**(n=8)**	**(n=8)**		
Median	560	1225	2.52	0.01
IQR	460-617	1148-1310		

n:number of valid analyzed cases
Median and IQR is calculated for all observations

Table II: Comparison of the Periapical area(mm^2): measured at baseline and last follow-up visit for each group.

Descriptive statistics	Preop	Last follow up	Z value	*P* value
Group I: Control	**(n=8)**	**(n=8)**		
Median	84	32	-2.52	0.01
IQR	67-144	23-42		
Group II: PRF+HA	**(n=8)**	**(n=8)**		
Median	82	23	-2.52	0.01
IQR	73-101	10-28		
Group III: PRF+HA+GTR	**(n=8)**	**(n=8)**		
Median	112	20	-2.52	0.01
IQR	107-170	18-25		

n:number of valid analyzed cases
Median and IQR is calculated for all observations

Table III: Comparison of the difference of bone density of the lesions (Δ HU) of the three groups at last follow-up period with respect to the pre-operative bone density

Descriptive statistics	Group I: Control (*n*=8)	Group II: PRF+HA (*n*=8)	Group III: PRF+HA+GTR (*n*=8)	H value	*P* value	Pairwise comparison (Groups)			
						Inferential statistics	I vs II	I vs III	II vs III
Median	288	550	699	7.21	0.03	Z value	-0.96	-2.65	-1.70
IQR	194-616	368-606	562-909			*P* value*	1.00	0.02	0.27

n:number of valid analyzed cases
Median and IQR is calculated for all observations
*Significance values have been adjusted by the Bonferroni correction for multiple tests.

Table IV: Comparison of the difference of radiolucent area of lesions(Δ mm^2) of the three groups at last follow-up period with respect to the pre-operative value

Descriptive statistics	Group I: Control (*n*=8)	Group II: PRF+HA (*n*=8)	Group III: PRF+HA+GTR (*n*=8)	Hvalue	*P* value	Pairwise comparison (Groups)			
						Inferential statistics	I vs II	I vs III	II vs III
Median	59	62	95	8.35	0.02	Z value	-0.25	-2.37	-2.62
IQR	40-94	43-81	83-151			*P* value*	1.00	0.05	0.03

n:number of valid analyzed cases
Median and IQR is calculated for all observations
*Significance values have been adjusted by the Bonferroni correction for multiple tests.

IV. Discussion

The early bone regeneration in apicoectomised tooth with large periapical lesion is required to provide functional support to the tooth. If required, orthodontic treatment can also be done only after the healing of the wound.

Inadequate bone healing results due to in-growth of connective tissue into the bony defect, preventing osteogenesis. In order to prevent this soft-tissue in-growth, bone grafts,GTR membrane are used to fill the bony space created after periapical lesion elimination by surgery.[15] However, true regeneration is not achieved with HA because, healing which occurs is just a connective tissue encapsulation of the graft.[16]

Clinical trials suggest that the combination of bone graft along with the growth factors in the PRF may be suitable to enhance the bone density.[17] PRF is a rich source of platelet derived growth factor (PDGF), transforming growth factor β (TGF-β), insulin like growth factor (IGF), vascular endothelial growth factor (VEGF), Epidermal Growth Factor (EGF) etc.

PDGF-promotesangiogenesis, activatesmacrophages that initiate the release of growthfactors from host tissue which enhances bone,cementum, and periodontal ligament repair andregeneration.[18,19]

TGF-β activates fibroblasts to induce collagen(type-I) formation, endothelial cells for angiogenesis,chondroprogenitor cells for cartilage andmesenchymal cells to increase the population ofwound healing cells.[20]

IGF-I stimulates bone formation by proliferationand differentiation[21], and it is synthesized andsecreted by osteoblasts.[22]

VEGF also promotes angiogenesis, increasesvascular permeability, stimulates mitogenesis forendothelial cells.[23]

EGF stimulates endothelial chemotaxis/angiogenesis, regulates collagenase secretion andepithelial /mesenchymal mitogenesis[23]

An increase in the proliferation of human osteoblasts has been demonstrated with a combination of PDGF, IGF-I, TGF, and EGF.[24]

Basal cells of oral epithelium are attached to basement membrane (BM) through hemi-desmosomal attachment. These cells proliferate due to inflammation and benign hyperplasia. BM prevents the passage of these cells to deeper layer of connective tissue. However, malignant epithelial cells produce proteolytic enzymes like matrix metalloproteinases, collagenases which destroy the basement membrane comprising of basal lamina (laminin, Type IV collagen) and reticular lamina (Type III collagen) and then occupy the underlying connective tissue.[25] So, epithelial cells in the mucoperiosteal flap in periapical surgery are not capable to infiltrate into the bony defect even when barrier membrane is not used.

It is generally believed that fibroblasts move faster than osteoblasts to occupy the bony defect left after apical surgery and thus consequently, a scar tissue is formed in large bone defects.[26,27] Barrier membranes are thought to prevent movement of proliferating fibroblasts from the surrounding tissue into the bony defect.[28,26,27] These osteoblasts do not have to compete with fibroblasts from surrounding tissue to occupy the surgical bony crypt. This allows regenerative cells of bone, PDL, cementoblasts to repopulate the area.[3]

Guided tissue regeneration (GTR) is an updated technique (s) aimed to enhance and direct cell growth for regeneration of specific periodontium tissues that have been damaged by either periodontal or pulpal diseases. Animal studies[29] also showed complete bone filling of periapical bone cavities after endodontic surgery, only when a barrier for guided tissue regeneration was used, whereas extensive connective tissue filling of the defects was found after conventional endodontic surgery.

Thus, the rationale for using GTR barrier membrane with bone grafting materials and PRF in the present study is to encourage the growth of surrounding tissue, while excluding unwanted cell types such as epithelial cells and connective tissue fibroblasts,asthis allows regenerative cells of bone, PDL, cementoblast to repopulate the area.[3]

In the present study, when pre-operative and post – operative (last follow up) increase in bone density and was high compared to Blood clot group andand decrease in lesion size were compared for intra-group comparisons, the difference was statistically significant.

The increase in bone density (at last follow up visit) of three study groups was analyzed and it showed a statistically significant difference among them. PRF+HA+GTR group showed highest value and was significantly high compared to control (blood clot) group.

On analysis of the decrease in periapical lesion area of three study groups, it showed a statistically significant difference among them and this decrease in lesion size was highest in PRF+HA+GTR Gr followed by PRF+ HA Gr and Control Gr. This difference in the latter two groups was also statically significant compared to the former one.

Thus, it can be stated that the bony healing evident through increase in bone density and decrease in lesion size was highest when the bony cavity was filled with PRF+HA along with application of GTR

membrane over it lowest when the bony cavity was left empty to be filled naturally with blood clot whereas intermediate in cases of combination of HA and PRF.

That the bony healing (decrease in lesion size and increase in bone density) was seen enhanced in HA + PRF mixture in the present study, is in accordance with the study conducted by Eldibany*et al.*, (2014) [30] in which only HA and PRF was taken in 15 patients kept under follow up for 9 months only.

Uppada*et al.* (2017) [31]in their study on two teeth observed through IOPAR that the mixture of HA+PRF along with amnion membrane enhanced bone regeneration through GTR technique.

However, a 2D and 3D study[32]for assessment of the effect of guided tissue regeneration using resorbable collagen membrane in the healing of through and through periapical lesions expressed that "periapical surgery with or without GTR was a predictable and viable solution for through and through lesions and there was no benefit in using a collagen membrane with regard to the outcome of periapical surgery in through and through lesions".

Impact of PRF application on healing of periapical bony defects after endodontic micro surgery after one year had been shown in the study of Ahmed El-Kabbaney*et al.*(2021).[33]

The role of HA in combination with PRF in enhancing bone regeneration in apicectomised teeth was also emphasized in the study of Thanikasalam M. *et al.*, 2018.[8]

But literature search is in dearth regarding CBCT comparison of healing potential of blood clot alone, HA+ PRF mixture and HA+ PRF mixture with GTR membrane over it in the bony crypt after periapical surgery which had been performed successfully probably for the first time in the present study.

V. Conclusion

Thus, within the limitations and the parameters of the present study, it can be concluded that the evidences of improvement in bone healing highlights the use of PRF+HA combination with resorbable GTR membrane as a valid method in inducing and accelerating hard tissue regeneration in apicoectomies teeth. However, more clinical studies with larger sample sizes and longer followed period coupled with favorable histologic evidences are required for definite conclusion

References

[1]. Lin L, Chen MY, Ricucci D, Rosenberg PA. Guided tissue regeneration in periapical surgery. J Endod 2010; 36:618-25.
[2]. Bosshardt DD, Sculean A. Does periodontal tissue regeneration really work? Periodontol 2000 2009; 51:208-19.
[3]. BashutskiJD,WangHL.Periodontal and endodontic regeneration. JEndod 2009Mar;35:321-8.Doi:10.1016/j.joen.2008.11.023.PMID:19249588
[4]. Melcher AH. On the repair potential of periodontal tissues. J Periodontol 1976;47: 256–60
[5]. Kenney EB, Lekovic V, Han T, Carranza FA Jr, Dimitrijevic B. The use of a porous hydroxylapatite implant in periodontal defects. I Clinical results after six months. J Periodontol1985;56:82-8.
[6]. Bowen JA, Mellonig JT, Gray JL, Towle HT. Comparison of decalcified freeze-dried bone allograft and porous particulate hydroxyapatite in human periodontal osseous defects. J Periodontol1989;60:647-54.
[7]. Saluja H, Dehane V, Mahindra U. Platelet-rich fibrin: A second generation platelet concentrate and a new friend of oral and maxillofacial surgeons. Ann MaxillofacSurg2011;1:53-7.
[8]. Thanikasalam M, Ahamed S, Narayana SS, Bhavani S, Rajaraman G. Evaluation of healing after periapical surgery using platelet-rich fibrin and nanocrystalline hydroxyapatite with collagen in combination with platelet-rich fibrin. Endodontology. 2018 Jan 1;30(1):25
[9]. Simonpieri A, Del Corso M, Sammartino G, Dohan Ehrenfest DM. The relevance of Choukroun's platelet‐rich fibrin and metronidazole during complex maxillary rehabilitations using bone allograft. Part II: Implant surgery, prosthodontics, and survival. Implant Dent 2009;18:220‐9.
[10]. Prasanthi NN, Chittem J, Simpsy GS, Sajjan GS. Surgical management of a large inflammatory periapical lesion with platelet-rich fibrin. J Interdiscip Dentistry 2017; 7:76-9.
[11]. Dahlin C, Linde A, Gottlow J, Nyman S. Healing of bone defects by guided tissue regeneration. PlastReconstrSurg1988;81:672–6.
[12]. Sculean A, Nikolidakis D, Schwarz F. Regeneration of periodontal tissues: Combinations of barrier membranes and grafting materials-biological foundation and preclinical evidence: A systematic review. J Clin Periodontol 2008; 35:106-16.
[13]. Pradeep AR, Bajaj P, Rao NS, Agarwal E, Naik SB. Platelet-rich fibrin combined with a porous hydroxyapatite graft for the treatment of three-wall intrabony defects in chronic periodontitis: A randomized controlled clinical trial. J Periodontol 2012; 83:1499-507.
[14]. J. Choukroun, F. Adda, C. Schoeffler, A. Vervelle, Une opportuniteenparo -implantologic: le PRF, Implantodontic 42 (2001) 55-62 (in French).
[15]. Nyman et al. 1980, 1982a, Karring et al. 1980. The guided tissue regeneration principle in endodontic surgery: one-year postoperative results of large periapicallesions. InternationalEndodontic Journal (l995) 28. 41-46
[16]. Shivashankar VY, Johns DA, Vidyanath S, Sam G. Combination of platelet rich fibrin, hydroxyapatite and PRF membrane in the management of large inflammatory periapical lesion. Journal of conservative dentistry: JCD 2013 May; 16(3):261.
[17]. Sunitha Raja V, Munirathnam Naidu E. Platelet-rich fibrin: Evolution of a second- generation platelet concentrate. Indian J Dent Res 2008; 19:42-6
[18]. Chung CP, Kim DK, Park YJ, Nam KH, Lee SJ. Biological effects of drug-loadedbiodegradablemembranesforguidedboneregeneration.JPeriodontalRes1997;32:172-5.
[19]. Dereka XE, Markopoulou CE, Vrotsos IA. Role of growth factors on periodontalrepair.Growth Factors 2006;24:260–7.
[20]. Monga P, Grover R, Mahajan P, Keshav V, Singh N, Singh G. A comparative clinicalstudy to evaluate the healing of largeperiapical lesions using platelet-rich fibrin andhydroxyapatite.Endodontology. 2016 Jan1;28(1):27

[21]. .Hock JM, Centrella M, Canalis E. Insulin-like growth factor I has independenteffectsonbonematrixformationandcellreplication.Endocrinology1988;122:254-60.
[22]. Baker NL, Carlo Russo V, Bernard O, D'Ercole AJ, Werther GA. Interactionsbetween bcl-2 andthe IGF systemcontrol apoptosis in the developing mousebrain.Brain Res Dev Brain Res 1999;118:109-18.
[23]. SmithR.,Gassmann,C,andCampbellM.Platelet-richPlasma:PropertiesandClinicalApplications.(2007);vol-2(2):73-78
[24]. Piché JE, Graves DT. Study of the growth factor requirements of human bone- derived cells: A comparison with human fibroblasts. Bone 1989; 10:131-8.
[25]. Hotary K, Lin X-Y, Allen E, *et al.*. A cancer cell metalloproteinase regulates the basement membrane transmigration program. Genes Dev. 2006; 20:2673–86
[26]. Dahlin C, Gottlow J, Linde A,Nyman S. Healing of maxillaryand mandibularbone defects using a membrane technique: an experimental study in monkeys.ScandJ PlastReconstr Surg Hand Surg. 1990;24: 13–9.
[27]. Pecora G, Baek S-H, Rethnam S, Kim S. Barrier membrane techniques in endodontic surgery. Dent Clin N Am. 1997; 41:585–602.
[28]. Dahlin C, Linde A, Gottlow J, Nyman S. Healing of bone defects by guided tissueregeneration.PlastReconstr Surg. 1988;81:672–6.
[29]. Divya S., Deepika P. C., Ambikathanaya. Platelet Rich Fibrin & Guided Tissue Regeneration Aided Coverage of a Mucosal Fenestration – An Interdisciplinary Approach, International Journal of Current Research and Review.
[30]. Eldibany RM, Shokry MM. The effect of Nanobone® in combination with platelet rich fibrin on bone regeneration following enucleation of large mandibular cysts. Tanta Dental Journal. 2014 Aug 1;11(2):100-8.
[31]. Uppada UK, Kalakonda B, Koppolu P, Palakurthy K, Manchikanti V, Prasad S, Samar S, Swapna LA. Combination of hydroxyapatite, platelet rich fibrin and amnion membrane as a novel therapeutic option in regenerative periapical endodontic surgery: Case series. International journal of surgery case reports. 2017 Jan 1;37:139-44.
[32]. P. D. Parmar,R.Dhamija,S. Tewari,P. Sangwan,A. Gupta,J. Duhan,S. Mittal.2D and 3D radiographic outcome assessment of the effect of guided tissue regeneration using resorbable collagen membrane in the healing of through-and-through periapical lesions – a randomized controlled trial..International Endodontic Journal,British endodontic society. 2019
[33]. Ahmed El-Kabbaney*et al.*. Impact of platelet Rich Fibrin Application on Healing of Periapical Bony Defects After Endodontic Peri-radicular Microsurgery :A One –Year Clinical study, AL-AZHAR Dental Journal ,2021

Shukdeb Mandal, et. al. "Comparative Evaluation of Healing After Periapical Surgery Using Hydroxyapatite Crystals and Platelet Rich Fibrin with and without Guided Tissue Regeneration Membrane:A CBCT Study." *IOSR Journal of Dental and Medical Sciences (IOSR-JDMS)*, 21(10), 2022, pp. 47-58.