International Journal of Current Advanced Research
ISSN: O: 2319-6475, ISSN: P: 2319-6505, Impact Factor: 6.614
Available Online at www.journalijcar.org
Volume 9; Issue 04 (A); April 2020; Page No.21831-21844
DOI: http://dx.doi.org/10.24327/ijcar.2020.21844.4300

Research Article

INJECTABLE PLATELET RICHFIBR IN (I-PRF) – A "WONDER" IN PERIODONTAL THERAPY

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A R T I C L E I N F O

Article History:
Received 14th January, 2020
Received in revised form 29th February, 2020
Accepted 05th March, 2020
Published online 28th April, 2020

Key words:

Periodontal regeneration, infrabony defects,I-PRF, DFDBA, bone regeneration, CBCT.

A B S T R A C T

Periodontal regeneration is defined as the reproduction or reconstitution of a lost or injured part to restore the architecture and function of the periodontium. The ultimate goal of periodontal therapy is to regenerate the lost periodontal tissues caused by periodontitis. For complete regeneration, delivery of growth factors in a local environment plays an important role as an adjunct to bonegrafts[50].Injectable Platelet rich fibrin (I-PRF) is considered as third-generation platelet concentrate, consisting of liquid concentrate of platelets, centrifuged at the lower centrifugation speed for lesser time with higher long-term releasing rate of various growth factors, easier manipulation with excellent healing properties. Hence, the aim of the present study is to investigate the clinical and radiological (bone fill) effectiveness of autologous I-PRF in combination DFDBA bone graft material and the DFDBA material alone in the treatment of intra bony defects.

INTRODUCTION

Periodontitis is the most ubiquitous infectious disease that leads to the destruction of the dental attachment apparatus, when not treated, results in progressive loss of the attachment apparatus that may eventually lead to early tooth loss. The main objective of periodontal treatment is the restoration of the periodontal cells infected by disease to their original form. This requires regeneration of the gingival connective tissues, cement genesis, regeneration of lost bone, re-attachment of connective tissue fibres into diseased root surfaces[1].

Periodontal bony defects are presented either in the form of horizontal or vertical pattern of bone loss. Defects with angular/intrabony defects have greater chances of bone regeneration if the contour of existing bone and the number of osseous walls isfavourable.[4]The regenerative potential of the periodontium invoked the flame to research various techniques and materials for the same, a number of surgical procedures alone or with bone grafts, guided tissue regeneration is employed.[1]

More recently several studies used PRF which is developed in 2001 by Choukroun, and has been termed as a second-generation platelet concentrate, PRF membrane releases multiple growth factor likePDGF, VEGF, IGF, EGF, and Beta FGF. The PRF promotes both bone augmentation and periodontal soft tissue regeneration functions by acting as a complex regenerative scaffold with progenitor specific mechanism.[43]I-PRF – Injectable Platelet Rich Fibrin is a newer advanced platelet enriched liquid "blood concentrate" of third generation with extended release of growth factors and enhanced stability of the graft by a 'solid' free of all movement's granules over the other PRF.The i-PRF demonstrated the ability to release higher concentrations of various growth factors and induced higher fibroblast migration and expression of PDGF, TGF-beta and collagen 1 and is used for better regenerative capacity. The Injectable PRF(i-PRF) will be obtained from the patient's blood and it would be expected that i-PRF treatment of infra bony defect may result in enhanced wound healing and periodontal regeneration.[70]

Today, Periodontal therapy widely use Demineralised freeze-dried bone allograft (DFDBA) due to its capacity to induce new bone formation, biocompatibility and as they exhibit both osteoconductive and osteoinductive properties which in turn leads to regeneration of hard and soft tissues of the periodontium but these bone graft material slack biomolecules for regeneration.[36] The potential of combining I-PRF with biomaterials for bone grafting creates an alternative to PRP as a platelet aggregate for bone regeneration. This method allows the use of the graft without the use of anticoagulants or other additives, thereby forming a well-agglutinated "steak for bone grafting".[63]

The hypothesis for this study is that i-PRF added with alloplastic bone graft material like DFDBA which in combination would attain optimal mechanical strength and would give more structural strength and stability to the graft till regeneration occurs. In this study, regeneration will be

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assessed by clinical parameters and by radiographic methods [CBCT].

METHODOLOGY

Source of data

This study was conducted on the Patients visiting the Outpatient Department of Periodontics at A.J. Institute of Dental Sciences, Mangalore for the treatment of their periodontal condition. Selected patients were divided (by coin-toss method) into two experimental groups: Group I was treated by DFDBA with i- PRF; and Group II was treated by DFDBA alone.

Methods of collection of data (including sampling procedure, If any)

The study sample 28 infrabony defects. Both male and female patients with age ranging from 25-60 years were included. A brief case history were recorded for the patients taking part in the study. Prior to initiating the study, the patients were informed of the purpose of this clinical trial and were requested to sign an informed consent. Ethical clearance was taken for the study.

Inclusion Criteria

1. Males and females aged between 25 and 60 years.
2. Diagnosis of chronic periodontitis based on AAP1999 Classification.
3. Chronic Generalised Periodontitis patients with clinical probing depth ≥ 6mm.
4. The presence of infrabony defects on CBCT (2/3 wall confirmed upon surgical exposure).
5. Patients willing to comply with multiple recall schedules.

Exclusion Criteria

1. Patients who have undergone any periodontal, surgical or nonsurgical therapy for past 6 months.
2. Patients who have received antibiotics and anti-inflammatory drugs in the past 4-6weeks.
3. Patients with a history of any systemic diseases or condition which influence the periodontal healing.
4. Patients with a habit of smoking, tobacco chewing and alcohol consumption.
5. Pregnant and lactating women.
6. Teeth which have Mobility, Recession and furcation involvement.
7. Patient who have taken Medication influencing bone Metabolism.

Presurgical Therapy

- ✓ Oral hygiene instructions and motivation of patients in performing effective oral
- ✓ hygiene measures.
- ✓ Nonsurgical periodontal therapy will be carried out by means of conventional scaling
- ✓ and root-planing using ultrasonic instruments and Gracey's area specific Curettes.
- ✓ Treatment of carious lesions and extraction of hopeless teeth.
- ✓ Anatomic factors are considered (Correction of overhanging restoration, malpositioned teeth, plunger cusps)

Presurgical Measurements

All the Patient were reviewed two weeks after Phase I therapy and the following clinical and radiological parameters were assessed.

- ✓ Full mouth Plaque Index (Silness and Loe,1964)
- ✓ Full mouth Gingival Index (Loe and Silness ,1963)
- ✓ Periodontal Probing Depth (PPD)
- ✓ Clinical Attachment Loss (CAL)
- ✓ Radiological assessment of infrabony defect using CBCT
- ✓ IBD-distance from CEJ to base of the defect

Clinical measurements were noted at baseline, 3 months and 6 months following surgery, radiographical measurement was noted at baseline and at the interval of 6 months. The customised acrylic stent was prepared on the study model for each patient using light cured acrylic to fit every selected patient. Vertical groove was made on the stent at the defect site which guided the placing of the Williams periodontal probe. This provided reproducibility for probing site.

Protocol for Preparation of I-PRF

The equipment required for PRF preparation includes an R-8C table centrifuge and a blood collection kit consisting of a 24-gauge needle and 10 ml blood collection tubes. A sample of blood were drawn from patient's cubital fossa and collected without anticoagulant in 10 ml tubes which were immediately centrifuged at a rate of 700 rpm for 3 minutes. After centrifugation, the resultant product will be consisting of three layers. The topmost layer consisting of acellular PPP (platelet poor plasma), PRF clot in the middle (orange colour area) and RBCs at the bottom of the test tube. The tube should then be opened carefully, to avoid homogenization of the material. Then slowly a 20 ml syringe with 18G hypodermic needle is inserted to the PRF clot in the middle of the tube and the liquid PRF (i-PRF) is aspirated into the syringe and the attached red blood cells in the bottom of the tube are scraped off and discarded.[10]

i-PRF Agglutination with Bone Graft

- ✓ Collected i-PRF were dispensed in a metal bowl.
- ✓ DFDBA will be added slowly into this bowl within 15 mins.
- ✓ After polymerization Graft were ready for placement at the defect site forming a well agglutinated "steak for bone grafting".

Surgical Procedure

- ✓ Following the administration of 2% lignocaine with 1:100,000 epinephrine.
- ✓ Depending on the quadrant of defect Nerve Block will be chosen.
- ✓ Sulcular Incision were made following the Gingival margin.
- ✓ Elevation of Mucoperiosteal flap allows proper debridement with the Gracey's area specific curette.
- ✓ Saline irrigation were done followed by placement of DFDBA graft with i-PRF in the defect in the group I and DFDBA alone in the group II. The Graft were placed till the crest of alveolar bone.
- ✓ Flap closure will be obtained by 4-0 Vicryl simple interrupted suture.
- ✓ The Periodontal dressing is placed.

- ✓ Post-surgery medication were prescribed with Antibiotics and Anti-inflammatory drugs.
- ✓ Periodontal dressing and sutures were removed after two weeks.
- ✓ Oral hygiene maintenance recommended.
- ✓ chlorhexidine mouthwash (0.2%) were recommended twice daily for two weeks.
- ✓ The Patient were instructed not to brush the area for two weeks post-surgery.

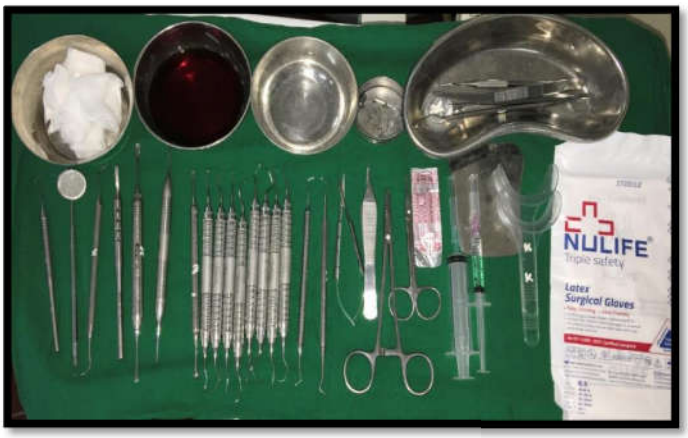

Fig 1 Surgical Armamentarium

Fig 2 Collection of Venous Blood

Fig 3 Centrifugation

Fig 4 After Centrifugation

Fig 5 Aspiration of I-Prf Using 20 Gauze Needle

Fig 6 'DFDBA' Bone Graft

Fig 7 I-Prf Mixed With DFBDA

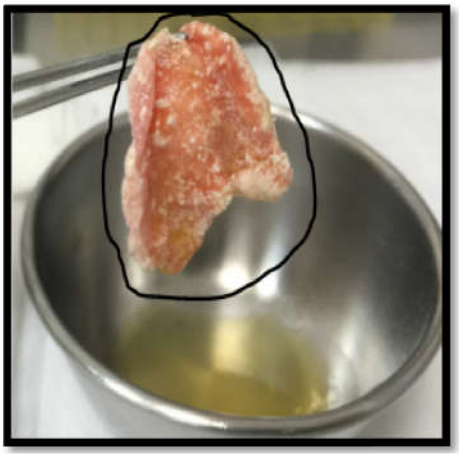

Fig 8-I-PRF Agglutination with DFDBA

Group I :Injectable Platelet Rich Fibrin With DFDBA

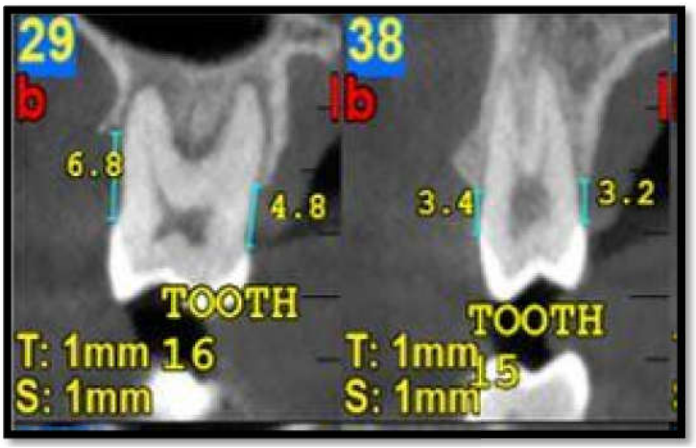

Fig 9 PRE-OP CBCT IBD Measurements

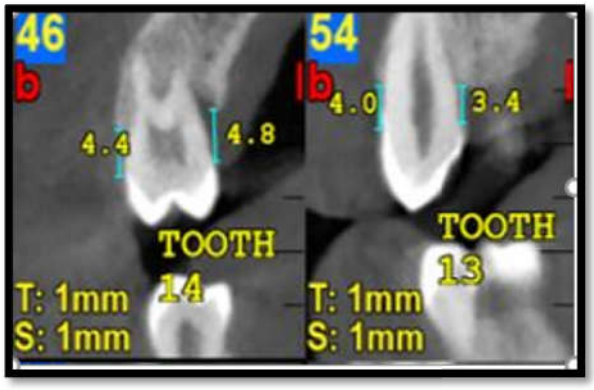

Fig 10-PRE-OP CBCT IBD Measurements

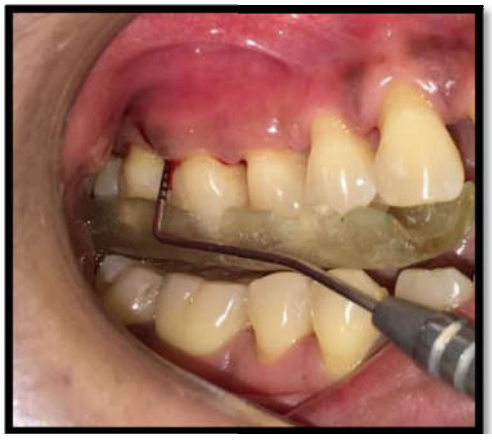

Fig 11 Baseline Measurement With Williams Periodontal Probe and Customized Stent

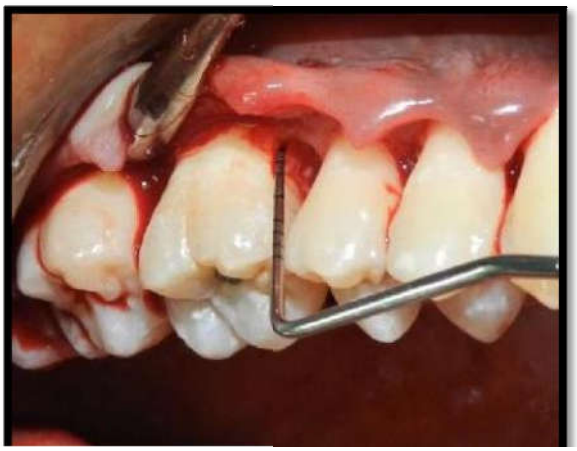

Fig 12 Flap Debridement

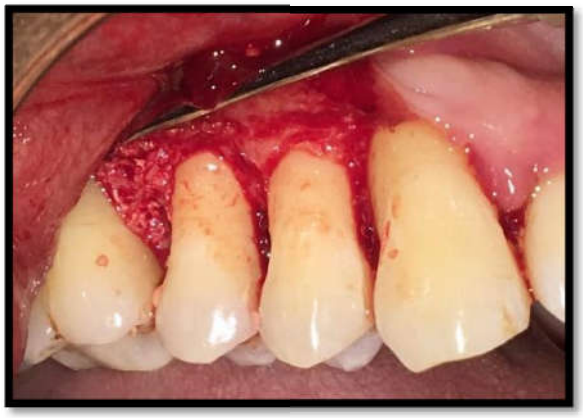

Fig13- DFDBA +I-PRF Placed In The DEFECT

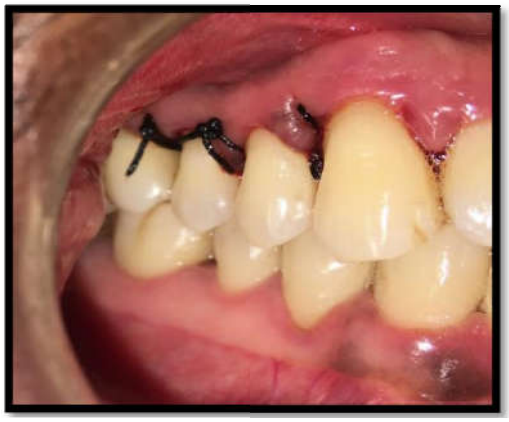

Fig 14- Suture Placement

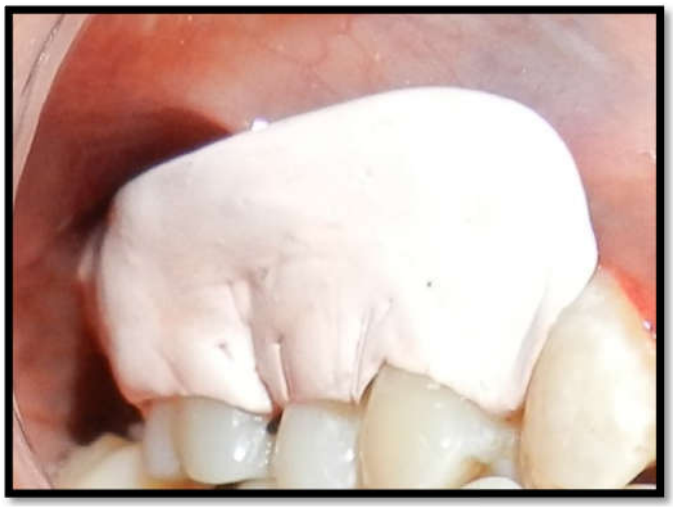

Fig 15 periodontal Pack Placed

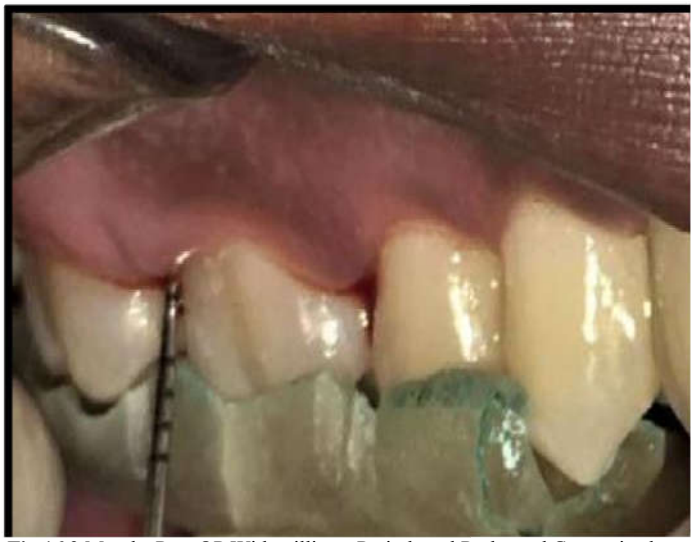

Fig 16 3 Months Post OP With williams Periodontal Probe and Customized Acrylic Stent

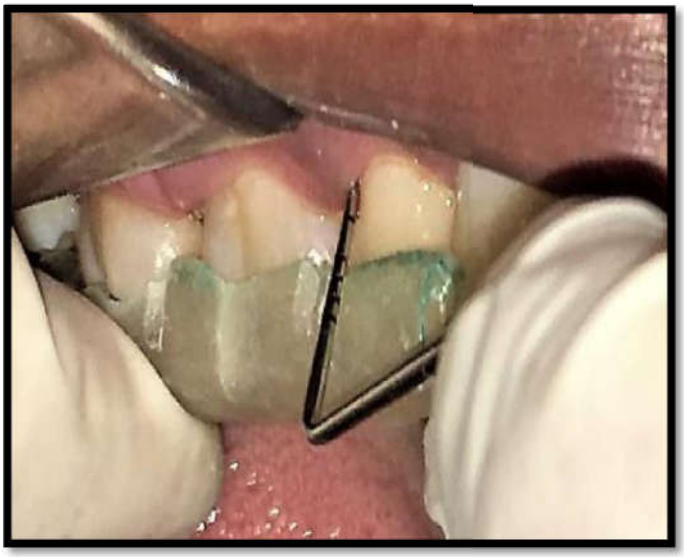

Fig 17- 6 Months Post op With Williams Periodontal Probe and Customized Stent

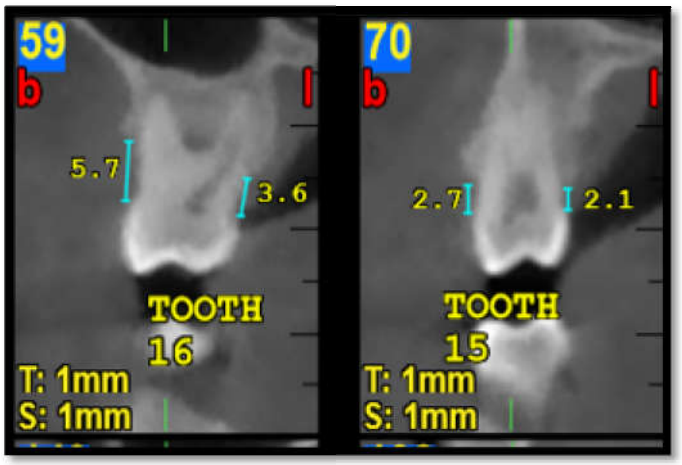

Fig18-Post OP 6 Months CBCT IBD measurements

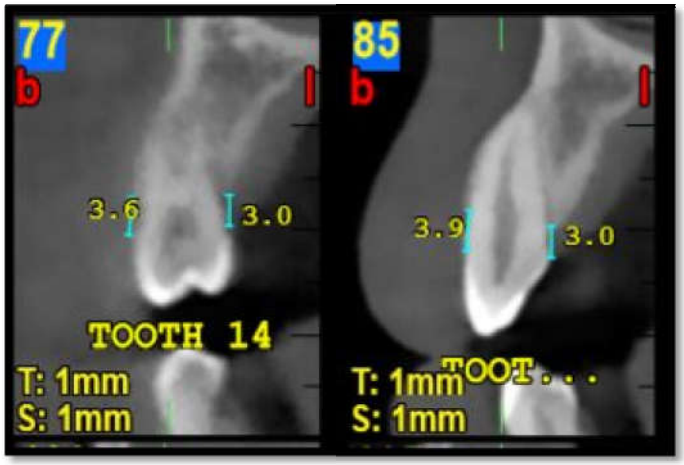

Fig 19 Post OP 6 Months CBCT IBD measurements

Group 2 Dfdba Alone

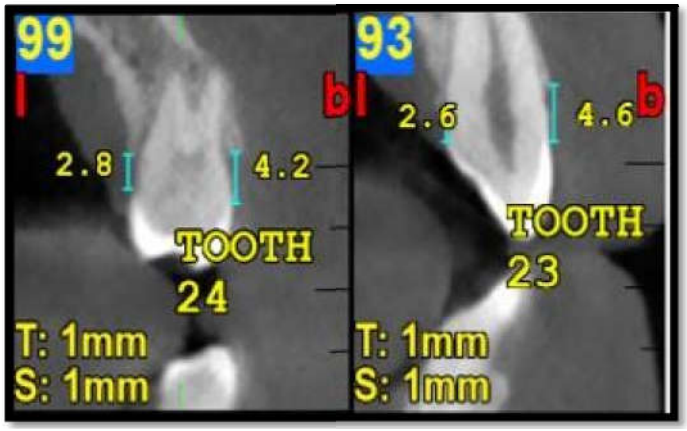

Fig 20-Pre-OP CBCT IBD Measurements

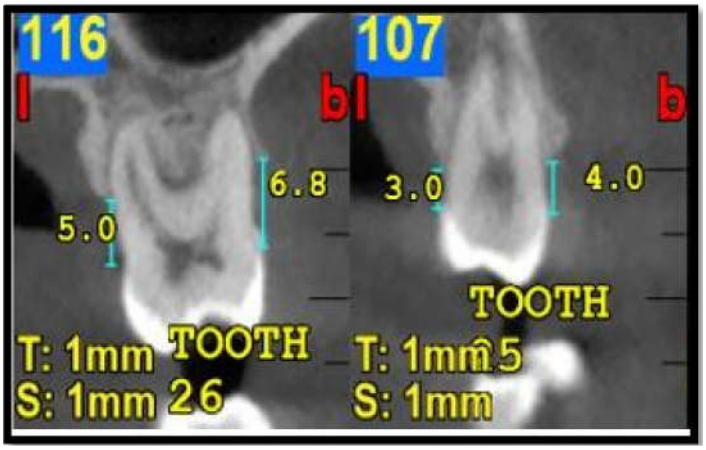

Fig 21-PRE-OP CBCT IBD Measurements

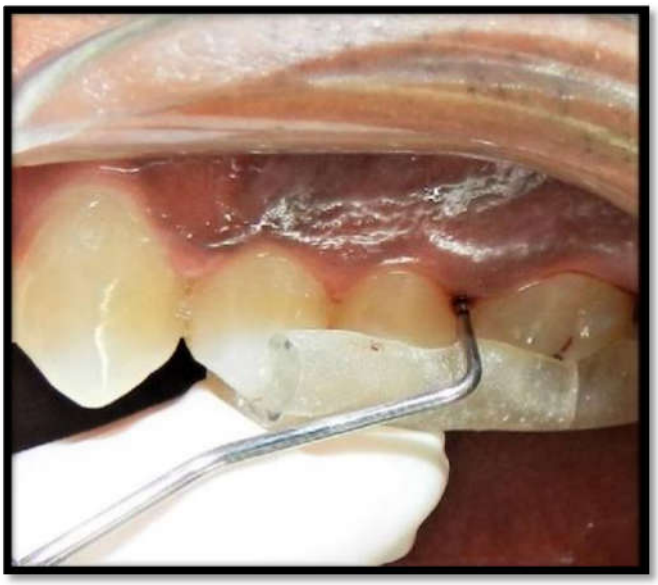

Fig 22- Presurgical Probing Depth With Williams Periodontal Probe and Customized Stent

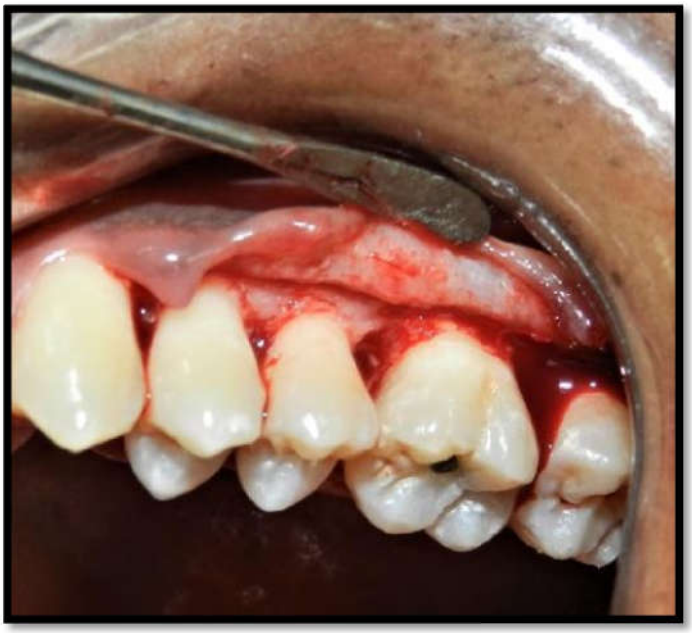

Fig 23- FLAP Debridement

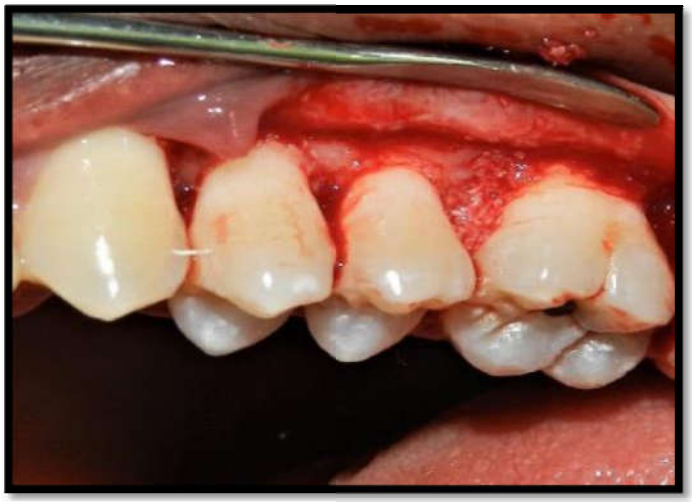

Fig 24- Placement OF DFDBA BONE Graft In To The Defect

Fig 25 Suture Placement

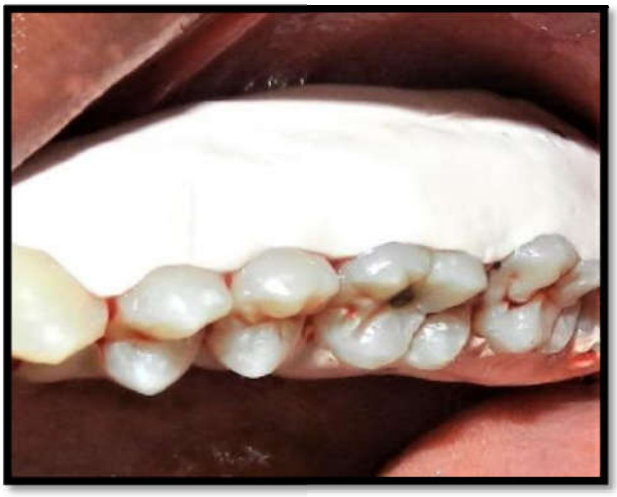

Fig 26- Periodontal Pack Placed

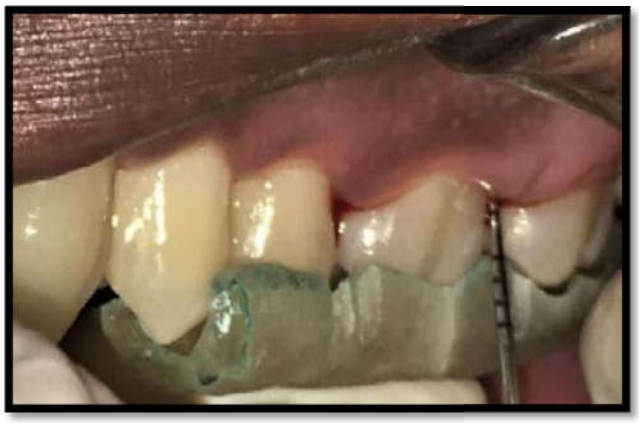

Fig 27- 3 Months Post op With Williams Periodontal probe and Customized Stent

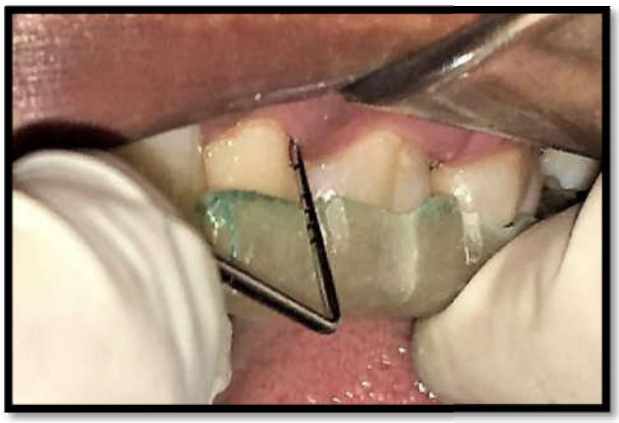

Fig 28- 6 months post OP with williams periodontal probe and customized stent

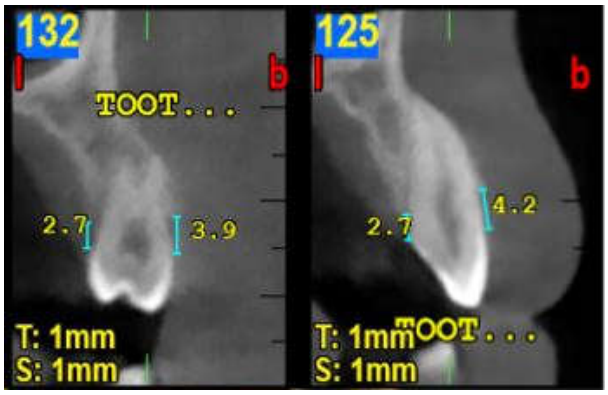

Fig 29 -Post- OP 6 Months Cbct IBD measurements

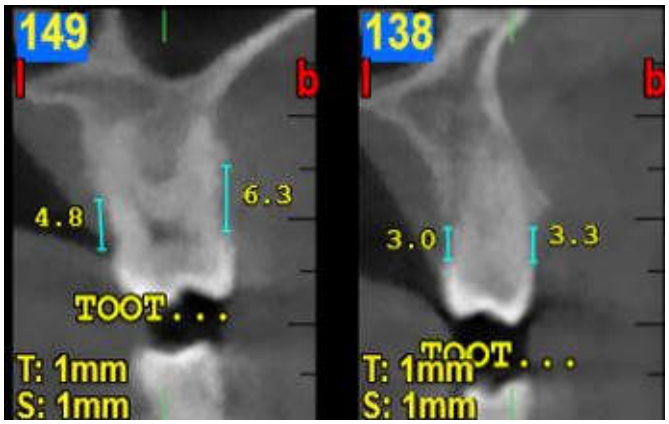

Fig 30-Post- OP 6 Months Cbct IBD measurements

Plaque Index

Group 1 (DFDBA + I-PRF)

The mean Plaque Index Value was 1.71 and 1.35 after 3 months and 6 months significant change in Plaque Index Values after 3 and 6 months of surgery in Group 1.

Table 1 Mean improvement in plaque index seen in group 1 at 3 months and 6 months

Group 1 (DFDBA + I-PRF)			
	Mean	**S.D.**	**SIG**
3 months	1.71	0.42	HS
6 months	1.35	0.51	HS

The mean Plaque Index was 1.64 and 1.35, at 3 months and 6 months respectively with P value at P<0.05 for all the months. Highly significant change was noticed in Plaque Index Values after 3 months and 6 months of surgery in group 2.

Table 2 Mean improvement in plaque index seen in group 2 at 3 months and 6 months. Plaque Index in Group 2 (DFDBA alone)

Group 2 (DFDBA alone)			
	Mean	**S.D.**	**SIG**
3 Months	1.64	0.48	HS
6 Months	1.35	0.51	HS

Comparative Evaluation/Analysis of the two groups with respect to Plaque Index

The mean difference of both Group 1 and 2 was 0.07 and 0 at 3 months and 6 months respectively with the P value 0.63, showing statistically no significant difference between the groups.

Table 3 Comparative Evaluation of Mean improvement in plaque index seen in group 1 and 2 at 3 months and 6 months.

GROUP 1 AND 2			
	Mean Difference	**P Value**	**SIG**
3 months	0.07	0.69	NS
6 months	0	1	NS

Gingival Index

Group 1 (DFDBA + I-PRF)

The mean Gingival Index improvement was 1.57 and 1.71 after 3 months and 6 months respectively with P value at P < 0.05 for both 3 and 6 months. Thus, there was highly significant change in Gingival Index values after 3 and 6 months of surgery in Group 1.

Table 4 Mean of gingival Index seen in Group 1 at 3 Months and 6 Months

GROUP 1			
	Mean	**S.D.**	**SIG**
3 months	1.57	0.42	HS
6 months	1.71	0.48	HS

Group 2

The mean gingival index improvement seen in Group 2 was 1.57 and 1.78 at 3 months and 6 months respectively with P value at P<0.05 for both 3 and 6 months. Highly significant change was noticed in gingival Index values after 3 months and 6 months of surgery in both the groups.

Table 5 Gingival Index seen in group 2 at Baseline, 3 months and 6 months

Group 2 (DFDBA alone)			
	Mean	**S.D.**	**SIG**
3 months	1.57	0.43	HS
6 months	1.78	0.49	HS

Comparative Evaluation/Analysis the Two Groups with respect to Gingival Index

The mean difference of both Group I and 2 was 0 and 0.07 at 3 months and 6 months respectively with the P value < 0.05 showing statistically no significant difference between the groups.

Table 6 Comparative Evaluation of Mean Gain in Gingival Index seen in group 1 and2 at 3 Months and 6 Months

	Group I and Group 2		
	Mean	**P Value**	**SIG**
3 months	0	1	NS
6 months	.07	0.67	NS

Probing Pocket Depth (PPD)

The results of the Probing Pocket Depth (PPD) values for each group are discussed below.

Group 1 (DFDBA + I-PRF)

The mean PPD reduction in Group 1 was 4.85mm and 5.71mm after 3 months and 6 months respectively with standard deviation of 1.63 and 1.89 respectively, t value 9.925 and 9.303 respectively and p value at P < 0.05 for both 3 and 6 months.

Thus, there was highly significant change in PPD after 3 and 6 months of surgery. The mean PPD reduction from 3 months to 6 months was 0.86 mm with the Standard deviation of 0.81, t value 5.0 and p value < 0.05 showing statistically significant result.

Table 7 Mean Probing Pocket Depth reduction in Group 1

	Group 1 (DFDBA + I-PRF)			
	Mean	**S.D.**	**T VALUE**	**SIG**
3 Months	4.85	1.63	9.925	HS
6 Months	5.71	1.89	9.303	HS
3 to 6 Months	0.86	0.81	5.000	S

Group 2 (DFDBA alone)

The mean PPD reduction was 4.07 mm and 4.64 mm after 3 months and 6 months respectively with the standard deviation of 1.43 and 1.74 respectively, t value 9.389 and 9.703 respectively and p value at P < 0.05 for both 3 and 6 months. Thus, there was highly significant change in PPD reduction after 3 and 6 months of surgery.

The mean PPD reduction from 3 months to 6 months was 0.57 mm with standard deviation of 0.52, t value 3.00 and p value <0.05 showing statistically significant result.

Table 8 Mean Probing Pocket Depthreductio n in Group 2

	Group 2 (DFDBA ALONE)			
	Mean	**S.D.**	**T VALUE**	**SIG**
3 months	4.07	1.43	9.389	HS
6 months	4.64	1.74	9.703	HS
3-6 months	0.57	0.52	3.000	S

Comparative Evaluation

Comparison of Group 1 and Group 2 at 3 months: The mean pocket depth reduction was 0.78 mm with standard deviation of 0.2 and p value < 0.05 statistically significant results on intergroup comparison.

Comparison of Group 1 and Group 2 at 6 months: The mean PPD reduction was1.07mm with standard deviation of 0.516, p value<.05 statistically significant results in favour of group 1 on intergroup comparison.

Table 9 Comparative Evaluation of Mean Probing Pocket Depthreductionin Group 1 and 2

	Group I and Group 2			
	Mean	**S.D.**	**P Value**	**SIG**
3 months	0.78	0.2	0.03	S
6 months	1.07	0.516	0.01	S
3-6 months	0.71	0.3	0.29	NS

Conclusion: Mean probing pocket depth reduction is greater in Group 1 as compared to Group 2 at both 3 and 6 months. The rate of reduction of mean probing pocket depth is faster when a combination of DFDBA + I-PRF is used as in Group 1 as compared to use of DFDBA alone in Group2.

Clinical Attachment Level (CAL)

Group 1 (DFDBA + I-PRF)

The mean gain in the clinical attachment level was 4 mm and 6.71 mm at 3 months and 6 months respectively with standard deviation of 0.85 and 0.53 respectively and p value < 0.05 showing statistically highly significant results.

Group 1 from 3 to 6 months

The mean gain in the clinical attachment level was 2.71 mm with standard deviation of 1.12 and p value < 0.05 showing statistically significant results.

Table 10 Clinical Attachment Level gainin Group 1

Group 1 (DFDBA + I-PRF)			
	Mean	**S.D.**	**SIG**
3 months	4.00	0.85	HS
6 months	6.71	0.53	HS
3-6 months	2.71	1.12	S

Group 2 (DFDBA alone)

The mean gain in the clinical attachment level was 3.42 mm and 4.07 mm at 3 months and 6 months with standard deviation of 1.23 and 1.46 respectively and p value < 0.05 showing statistically highly significant results.

Group 2 from 3 to 6 months

The mean gain in CAL was 0.65 mm from 3 months to 6 months with standard deviation of 0.57 and p value < 0.05 showing statistically significant results.

Table 11 Clinical Attachment Level gain in Group 2

	Group 2 (DFDBA alone)		
	Mean	**S.D.**	**SIG**
3 months	3.42	1.23	HS
6 months	4.07	1.46	HS
3-6 months	0.65	0.57	S

Comparative Evaluation of Gain in CAL in Group 1 and 2 at 3 Months and 6 Months

The mean gain in the Clinical attachment level was 0.58 and 2.64 at 3 and 6 months respectively with standard deviation of 1.69 and 2.66 and p value >0.05 showing statistically non-

significant results on 3 months interval and p value < 0.05 showing statistically highly significant results on intergroup comparison.

Table 12 Comparative Evaluation of gain in CAL in Group 1 and 2 at 3 months and 6 months

Group I and Group 2				
	Mean	**S.D.**	**T Value**	**SIG**
3 months	0.58	1.69	1.21	NS
6 months	2.64	2.66	3.01	HS

Defect Fill AT 6 Months

Group 1(DFDBA + I-PRF)

The mean Defect fill in Group 1 was 1.77 with the standard deviation 0.58, showed
p value at P < 0.05 statistically highly significant results at 6 months.

Table 13 Mean Defect fill in Group 1 at 6 months

Group 1 (DFDBA +I-PRF alone)			
Mean	S.D.	T VALUE	SIG
1.77	0.58	8.112	HS

Group 2(DFDBA alone)

The mean Defect fill in Group 2was 1.02 with the standard deviation .42, showed p value at P < 0.05 statistically highly significant results at 6 months.

Table 14 Mean Defect fill in Group at 6 months

Group 2 (DFDBA alone)			
Mean	S.D.	T VALUE	SIG
1.02	0.42	8.836	HS

Group 1 and 2 comparisons

Mean defect fill was .75mm with the standard deviation .5944,showed pvalue < .001 statistically significant results favouring greater bone fill in the group 1 at 6 months interval.

Table 15 Comparative evaluation of mean defect fill in group 1 and 2 at 6 months

Comparision OF Group 1 And 2			
mean	**S.D.**	**P Value**	**SIG**
0.75	0.59	0.00020	HS

DISCUSSION

In this study, the use of an injectable formulation of PRF (termed i-PRF) has been pursued with the aim of delivering to an easy to use platelet concentrate in liquid formulation which can be either utilized alone or combined easily with various biomaterials forming a steak of bone graft after polymerisation of 10-12 mins, which can be easily moulded and condensed in to the bony defect . As this steak of bony graft sticks to the surgical site when placed, it has an ability to form an early physiologic "seal" with the host tissue precluding bacterial contamination.

I-PRF with a slower and shorter centrifugation speeds, has a high amount of regenerative cells with higher concentrations of growth factors when compared to other formulations of PRF [70] Furthermore, I-PRF is easy to manipulate and is free of graft movements during the suturing of the defects. I-PRF is shown have significantly higher levels of total long-term release of PDGF-AA, PDGF-AB, EGF and IGF-1 after 10days with high biocompatibility, higher fibroblast migration and high proliferation rate.[70]

I-PRF showed significantly highest mRNA levels of TGF-β at7 days, PDGF at 3 days, and collagen1 expression at both 3 and 7 days when compared to PRP. Various growth factors released from I- PRF have slower and sustained release up to 10 days and up to 28 days, which means I-PRF stimulates its environment for a longer duration during remodelling. Moreover, I-PRF increase cell migration, proliferation and collagen related protein expression of human osteoblasts.[70]
Another interesting finding is the fact that while PRF slowly dissolved over time, i-PRF forms a small clot likely as a result of fibrin components that acts as a dynamic gel with cells likely contained within its hydrogel. It is therefore hypothesized that even following 10 days, an additional release of growth factors could still be expected from i-PRF whereas PRF would basically dissolve entirely after 10 days.[70]
Morphological analyses revealed that the slow polymerization of i-PRF results in a three-dimensional fibrin network embedding platelets, leukocytes, type I collagen, osteocalcin and growth factors. Thus, i-PRF becomes a good approach as an injectable material to be associated with other biomaterials.
Thus, the human liquid fibrinogen in the i-PRF is slowly converted into fibrin that can act as an autologous fibrin binder (AFB). This technique has been recently used by clinicians to promote an agglomeration or coating of biomaterials to enhance wound healing.[69]

Studies have evaluated the release of different growth factors from i-PRF, as well as its influence on the behaviour of fibroblasts and osteoblasts.[69] Bioactivity, simplified preparation technique, and association with other biomaterials are key aspects to stimulate the use of i-PRF in the field of orthopaedics, periodontics, and implant dentistry.

Previous studies have shown cumulative effect when graft material was used with platelet rich fibrin (PRF) due to the concentrated suspension of the growth factors found in platelets. All of the known clinical applications of PRF highlight an accelerated tissue cicatrisation due to the development of effective neovascularization, accelerated wound closing with fast cicatricial tissue remodelling, and nearly total absence of infectious events. [23]

The combination of fibrins and cytokines within PRF becomes a powerful scaffold with an integrated reservoir of growth factors for tissue regeneration.[24] Besides promoting wound healing, bone growth and maturation, PRF with bone graft have the advantages of graft stabilization, wound healing, haemostasis and improved handling properties.[80] Thus in this clinical study we decided to use a combination therapy with the I-PRF + DFDBA .

The success of periodontal therapy is based on regular program of recall maintenance and oral hygiene instructions. Periodontal surgical therapy in the absence of an appropriate supportive periodontal therapy will fail eventually. According to the literature, six-month evaluation is considered to be standard time frame for evaluating the success of periodontal regeneration. [25]

Post-surgical evaluation by surgical re-entry after 6 to 12 months of graft placement was considered to have sufficient level of evidence ,Though the Surgical method is considered as the gold standard for evaluating regeneration in this study, we have used CBCT as the studies have shown that both techniques were equally effective in evaluating regeneration.[26]CBCT provides the 3 dimensional views of the

defect with high resolution imaging thereby overcoming the adverse effects of surgical re-entry in assessing the defect fill or bone gain.

The subjects recruited in this study had varied oral hygiene status which was brought down to minimal Plaque Index scores following scaling and root planing and the baseline values were maintained for all the three groups after the surgery at 3, 6 months.

All the subjects were on supragingival plaque maintenance program every month during the follow up period. Notably, patients in our study who failed to comply with the oral hygiene instructions and maintenance schedule, were found to have lesser amount of improvement in the clinical parameters and radiographic assessment.

The statistical result showed significant reduction in plaque index both in group 1 (The mean Plaque Index improvement was 1.71 and 1.35 at 3 and 6 months) and group 2 (1.64 and 1.35 at 3and 6 months) and p<0.05 for all the months in both the groups. The mean difference of both Group 1 and 2 was 0.07 and 0 at 3 months and 6 months respectively with the P value 0.63; showing p>0.05, statistically no significant difference between the groups. Bansal *et al* [44] showed reduction in mean plaque index from baseline to six months with no statistical difference in the intergroup comparison which is in accordance to the present study

There was statistically significant reduction in the gingival index seen both in group1 (The mean Gingival Index improvement was 1.57 and 1.71 after 3 months and 6 months) and group 2 (1.57 and 1.78 at 3 months and 6 months) respectively with P value at P < 0.05 for both 3 and 6 months. Thus, there was highly significant change in Gingival Index values after 3 and 6 months of surgery in Group 1and 2. The mean difference of both Group I and 2 was 0 and 0.07 at 3 months and 6 months respectively with the P value < 0.05 showing statistically no significant difference between the groups. These results are in accordance with the studies of Piemontese M *et al*[34]., Markou N *et al*[35]

Reduction in PD, IBD and gain in CAL are the major clinical outcomes measured to determine the success of any periodontal treatment. In the present study, Probing Pocket depth showed statistically significant reduction both in group 1(4.85mm, 5.71mm at 3 and 6 months) and group 2(4.07mm, 4.64 mm at 3 and 6 months) and group 1 showed significant reduction than group 2(0.78mm and 1.07mm at 3 and 6 months) and p value < 0.05 statistically significant results on intergroup comparison. The mean probing pocket depth reduction is greater in Group 1 as compared to Group 2 at both 3 and 6 months. The rate of reduction of mean probing pocket dep this faster when a combination of DFDBA + I-PRF is used as in Group 1 as compared touse of DFDBA alone in Group 2.The reduction in the probing depth in group 2 was comparable with results of the studies done by Melloning JT *et al*[21] , Bansal *et al*[44] where they used DFDBA alone to treat the infrabony defects. The results of group 1 showed similarity with the studies when treated with DFDBA +PRF by Bansal C *et al*,[44] Markou *et al*[35], Agarwal A *et al*[62]

Clinical attachment level gain was statistically significant both in group 1(4.00mm and 6.71mm at 3 and 6 months) and group 2(3.42mm, 4.07mm at 3 and 6 months) and group 1 showed significant reduction than group 2(0.58mm, 2.64mm at 3 and 6 months). The mean gain in the Clinical attachment level was 0.58 and 2.64 at 3 and 6 months respectively with standard deviation of 1.69 and 2.66 and p value> 0.05 showing statistically non-significant results on 3 months interval and p value < 0.05 showing statistically highly significant results on intergroup comparison showing better soft tissue gain in group 1.The results are in accordance with studies done by Agarwal *et al*[62] , Bansal *et al*[44] . Statistically significant bone defect fill (bone gain) is seen in both in the group 1 (1.77 mm) and the group 2 (1.02 mm).These results are similar to the studies done by Bansal *et al*, Guillemin MR *et al* On comparison of group 1 and group 2 Mean defect fill was .75mm with the standard deviation .5944, showed pvalue < .05 statistically significant results favouring greater bone fill in the group 1 at 6 months interval.

This supports the significance and advantage of various growth factors present in the I-PRF which accelerates the soft and hard tissue healing [79].

Most of the defects treated in the present study are three walled and two walled defects. One also has to consider that the potential for bone fill may differ depending on the morphology of the angular bone defect. Most angular defects appear as combinations of one-, two- and three-wall defects and whereas the two- and three-wall component of an angular bone defect may show great potential for bone fill during healing, the one-wall component will rarely demonstrate this type of healing.

In the meta-analysis done by Shah M *et al*[28] showed CAG from 3.03mm to 4.73, reduction in pocket depth in the range of 3.77 to 4.69mm and defect fill in the range of 1.93 to3.20mm which is in accordance with the present study

Two reviews by Laurell *et al*. (1998)[28]and Lang (2000)[29], reported the weighted mean bone defect fill in the angular defect by open flap debridement is 1.2mm which is in accordance with the present study.

The demonstration of the better intergroup comparison results between the groups in all the parameters and significant bone fill in I-PRF + DFDBA group in the present study may be explained by the additional biologic effects of the I-PRF. Slow polymerization of I-PRF results in a three-dimensional fibrin network embedding platelets, leukocytes, type I collagen, osteocalcin and growth factors. It progressively releases cytokines during fibrin matrix remodelling. Leukocytes seem to have a strong influence on growth factor release, immune regulation, anti-infectious activity during healing. It permits rapid angiogenesis and is an optimal matrix for migration of endothelial cells and fibroblasts. It can also be speculated that BMPs which are the members of TGF super family present in DFDBA will add to the effects of the growth factors within the platelets ensuring a synergetic impact on the cell population of the wound. Whereas the PD reduction and attachment gain in the DFDBA along group may be the result of new attachment apparatus, new cementum, new connective tissue and new bone formation.[34,40]

The literature includes no clinical studies using DFDBA in combination with I-PRF and DFDBA alone in the treatment of infrabony defects of the periodontium. So, in the present study we have used this combination. No discomfort or any other complications were observed at any of the treated sites of the defect.

Future research to further clarify the exact mechanisms for these differences remains necessary. In the future, there also remains great interest to continuously and steadily increase our understanding of advanced platelet concentrates and the role of the various cell types found within their formulations.

It is an important challenge for researchers working in regenerative dentistry to further characterize the potential of each platelet formulation on new bone formation and tissue wound healing and to further compare their regenerative potential by fully revealing their added advantages/disadvantages.

CONCLUSION

From the observations of this clinical study ,it can be concluded that I- PRF is efficacious clinically and radiographically in the treatment of periodontal infrabony defect .The treatment of intrabony defects with the combination of I-PRF and DFDBA resulted in significant improvements in both, clinical parameters at baseline, 3 months and 6 months and Radiographic parameters using CBCT at baseline and 6 months post surgery. DFDBA is shown to increase the clinical effects observed with I-PRF in the treatment of human intrabony defects. The use of autologous liquid injectable platelet preparations like I-PRF allows the clinician to optimize tissue remodelling, wound healing and angiogenesis by the local delivery of higher amount of growth factors and proteins although DFDBA material provide the required area for the formation of bone due to the nature of the space. According to the results obtained in this clinical study, it could be concluded that the positive clinical impact of additional application of I-PRF with DFDBA graft material in treatment of periodontal intrabony defect.

The use of I-PRF in the periodontal regeneration procedures would be both clinically effective and cost effective with no antigenicity. However, long term, multicentre randomized, controlled clinical trial will be required to know clinical and radiographical effect over bone regeneration. Also the long-term results associated with both modalities of therapy, as well as the histological nature of newly formed tissues by either treatment, remains to be elucidated.

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How to cite this article:

Dr. Kavyashree P.M and Dr. Nagarathna D.V (2020) 'Injectable Platelet Richfibrin (I-Prf) – A "Wonder" in Periodontal Therapy', *International Journal of Current Advanced Research,* 09(04), pp. 21831-21844.
DOI: http://dx.doi.org/10.24327/ijcar.2020.21844.4300

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