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Evaluation of the Effect of a Hydroxyapatite-Beta Tricalcium Phosphate (βTCP) graft with Platelet Rich Fibrin Occlusive Membrane on Intrabony Defects Accessed With a Single Flap Approach –A Randomized Controlled Clinical trial.

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ABSTRACT

The aim of this study was to evaluate and compare the clinical and radiographic outcomes of intrabony defects accessed with a Single Flap Approach, with an occlusive Platelet Rich Fibrin membrane, that did or did not receive an additional Hydroxyapatite - Beta Tri Calcium Phosphate composite bone graft. The study comprised 30 patients presenting with intra-bony defects who were randomly treated using Single Flap Approach with Platelet Rich Fibrin and with or without additional bone graft (Hydroxyapatite + β Tri Calcium Phosphate composite graft). Patients were recalled at 12 and 36 months to assess and compare the Full Mouth Plaque scores (FMPS%), Full mouth bleeding scores (FMBS%),Probing Pocket Depth (PPD), Relative Clinical Attachment Level (R-CAL),position of Gingival Margin at surgical site (GM), and Radiographic assessment of crestal bone level with Radiovisiography - RVG (mm). The results revealed a significant reduction in the mean FMPS%, FMBS%, & PPD within the groups ($p \leq 0.0001$),but did not reveal any statistical significance when compared between the test and the control groups at various time points (12 and 36 months). Both test and control groups showed statistically significant gain in the mean Relative Clinical Attachment Levels (R-CAL) at 12 months ($p \leq 0.0001$). The position of the gingival margin (GM) had a minimal gradual apical shift (gingival recession) till 12 months with a mild statistical significance of ($p \leq 0.048$) in the control group. The test group initially had a coronal shift at 12 months' time point and then shifted apically later and became comparable at 36 months between the control and the test group but with no statistical significance($p \leq 0.784$).RVG values revealed a statistically significant radiographic bone fill ($p \leq 0.0001$) in both, test group and the control groupat 12 months' time point. This Bone fill significantly improved in both the groups when measured at 36 months, and the test group had a better bone fill compared to the control group with statistical significance ($p \leq 0.023$).The results of this study indicate that the sites which received PRF alone and PRF + HA - βTCP in the treatment of periodontal intraosseous defects accessed with Single Flap A were clinically effective with a substantial probing pocket depth reduction and clinical attachment gain. Although the adjunctive use of HA -βTCP graft shows a significant bone fill radiographically under the experimental conditions it offers no significant adjunctive effects on the surrogate markers.

Keywords
Intrabony defects, PRF, HA -βTCP, single flap approach, periodontal flap, bone fill

Introduction

The ultimate goal of periodontal therapy remains the predictable three-dimensional repair of an intact and functional periodontal attachment apparatus that replicates its pre diseased structure. Most techniques that are purported to regulate lost periodontal tissues are either unpredictable or have an inherent capacity to heal, by repair of the periodontal tissues, rather than periodontal regeneration.[1]

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Over the years, minimally invasive periodontal surgical techniques are being developed with the intent to lessen surgical trauma, while providing limited, although adequate, surgical access, so that the tissues following manipulation may be primarily secured, thereby enabling periodontal wound stability.[2,3] Towards this end, the Single Flap Approach was developed which allows for minimization of soft tissue trauma and the removal of granulation tissue from periodontal defects using a much smaller surgical incision, that ensures wound and clot stability.[4,5,6]
Regenerative techniques that have emerged over the last few decades have rested on two basic tenets; the use of bone grafts, to regenerate the lost alveolar bone, and the use of Guided Tissue Regeneration (GTR) membranes to selectively repopulate the period with cells that arise from the periodontal ligament, to ensure the formation of a connective tissue attachment, on a previously diseased root surface.[7]
Recent publications have extrapolated the effects of PRF when used along with open flap debridement.[8] These studies have found that the additive use of PRF, increases Probing Pocket depth reduction and Clinical Attachment gain. The Single Flap Approach and the Platelet Rich Fibrin Matrix, both have the potential to positively alter periodontal wound healing.[9,10,11]

In the present study, we proposed to place an occlusive PRF membrane over a hydroxyapatite plus beta tricalcium phosphate composite graft into an intrabony defect that is accessed by a Single Flap Approach.We hypothesize that the concomitant use of these treatment strategies would have a synergistic effect on periodontal tissue regeneration. There are no earlier published studies with this design and this study is proposed to evaluate, the potential of this technique to promote optimal periodontal tissue regeneration.

Materials and Methods

Ethical clearance:

Institutional Scientific and Ethical Review Board granted the ethical clearance(IRB Approval no - SRMDC/IRB/2014/MDS/No.503).Surgical procedures including the possible risk, benefits and complications were explained and both verbal and written informed consent was obtained.

Subject recruitment & allotment:

Thirty six subjects with two/three walled infrabony defects were recruited into the study and underwent Phase 1 therapy. Six weeks later thirtytwosubjects reported for review, four subjects were lost to follow up. All the clinical parameters were to be assessed by a single calibrated examiner. Blood investigations and intra oral periapical radiograph using RVG were performed and final recruitment was done when they fulfilled the other selection criteria after phase I therapy.

Phase I Therapy:

Oral hygiene instructions and motivation of the patients.Non-surgical periodontal therapy was done by means of conventional scaling and root planing, using curettes and ultrasonic instruments.Treatment of carious lesions.Anatomic factors considered (e.g. correction of over - hanging restoration, malposed teeth).

Inclusion Criteria:

Systemically healthy patients with Probing pocket depth ≥5 mm and Clinical attachment loss of ≥ 3 mm after phase I therapy, Patients with 2 wall or 3 wall defect that are non - circumferential andpresence of FMPS ≤ 20%& FMBS ≤ 20%.However, Patients requiring antibiotic prophylaxis before the periodontal examination or under immunosuppressive drugs, diagnosed with malocclusion, Female patients with hormonal imbalance, currentsmokers and pan chewers were excluded.Allocation of the treatment option for each patient was on a random basis, patients under Group A received SFA with PRF and Group B received SFA with PRF and HA--TCP composite graft.
Patients were reviewed 6 weeks after phase I therapy and the following clinical parameters were assessed -

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1. Full Mouth Plaque scores. (O'Leary 1972) - FMPS%

2. Full Mouth Bleeding scores. (Ainamo and Bay 1975) – FMBS%

3. Assessment of Probing Pocket Depth at the surgical site. – PPD(mm)

4. Assessment of Relative Clinical Attachment Level at the surgical site – RCAL (mm)

5. Assessment of Relative Gingival Position at the surgical site – GM(mm)

6. Assessment of Radiographic Crestal Bone Level with RVG- RVG(mm)

Radiographic evaluation:

Digital radiographic assessment involved placement of the photo - stimulable phosphor plates on an x - ray positioning and holding device.The X-ray positioning and holding device was provided with a bite plane where the patients occlusal relationship was recorded with poly -vinyl siloxane material (heavy body impression material). A guiding arm attached to the X -ray positioning and holding device facilitated reproduction of the position of the tube head, with the same horizontal and vertical angulation, thereby standardizing the radiographic image. The PSP plates were mounted on the X -ray positioning and holding device and radiographs obtained.The PSP plates were scanned using DIGORA software and digital images were obtained.Crestal bone levels were measured at baseline and six month time points using SOPRO IMAGING software.Initially a horizontal line was created along the highest crestal bone level in the surgical site. Thereafter a vertical line drawn from the most deep crestal bone level perpendicular to meet the previously drawn horizontal line.The dimension of the vertical line was used to measure the crestal bone level at baseline and six months and compared.

PRF Preparation:

The PRF was prepared following the protocol developed by Choukroun et al.[8]Just prior to surgery, intravenous blood (by venipuncture of theantecubital vein) was collected in three 10-ml sterile tubes without anticoagulant and immediately centrifuged in centrifugation machineat 3,000 revolutions (approximately 400 g) per minute for 10 minutes. Blood centrifugation immediately after collection allows the composition of a structured fibrin clot in the middle of the tube, just between the red corpuscles at the bottom and acellular plasma (Platelet-poor plasma [PPP]) at the top. PRF was easily separated from red corpuscles base [preserving a small red blood cell (RBC) layer] using a sterile tweezerand scissors just after removal of PPP and then transferred onto a sterile compress. A stable fibrin membrane was obtained by squeezing serum out of the PRF clot.

Surgical Procedure:

Intra-oral antisepsis was performed with 0.12% chlorhexidine digluconate rinse and iodine solution was used to carry out extraoral antisepsis. Following administration of local anaesthesia, the single flap access was performed in the selected sites. Meticulous defect debridement and root planing were carried out using ultrasonic instrumentsand area specific curettes. No osseous recontouring was carried out. In the control group, an occlusive PRF matrix was grafted into the defect and in test group,the graft [A commercially available synthetic bone graft substitute/ regenerative material composed of HA-β-TCP composite graft (Ossifi™) of particle size 0.25-1 mm and 0.5 cc granules] is gently pushed into the intrabony defects followed by the occlusive PRF membrane.Care was taken not to overfill defects. Wound closure was obtained using vertical internal matrix sutures with vicryl -0 and the surgical area was protected and covered with periodontal dressing(COE – PAK).

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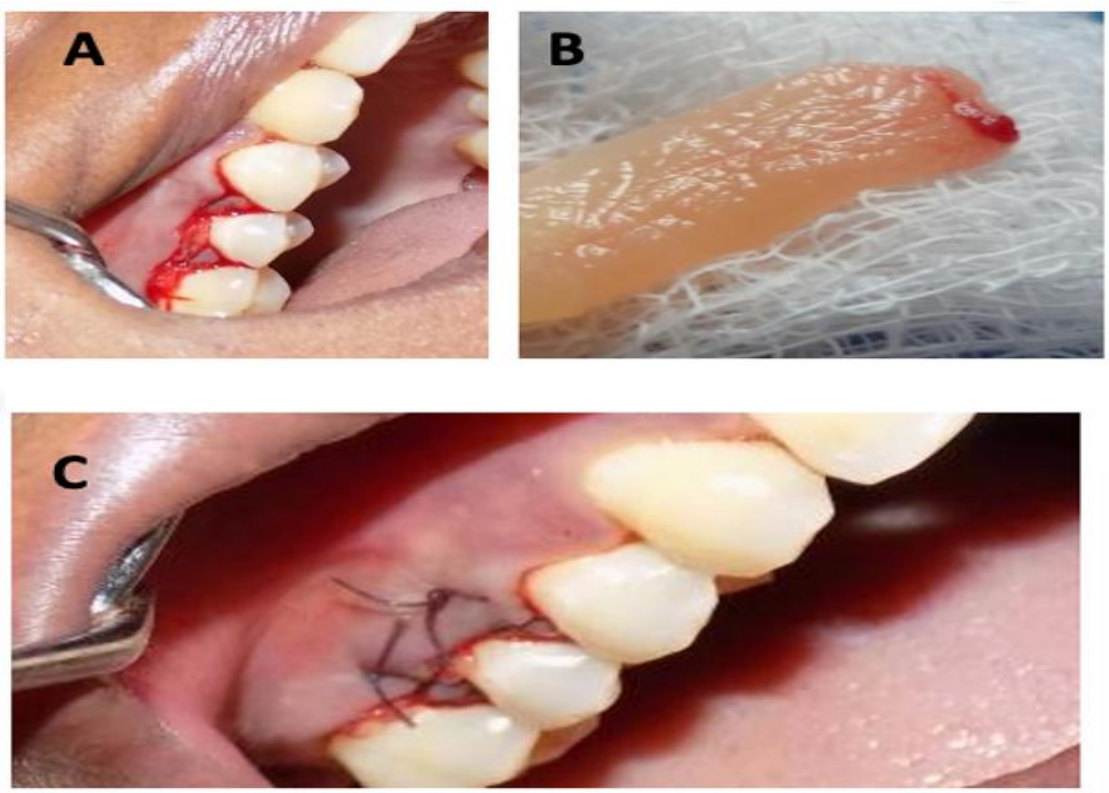

A.SINGLE FLAP ACCESS PERFORMED
B.PREPARATION OF PRF MATRIX
C.WOUND CLOSURE USING VERTICAL INTERNAL MATRIX SUTURES

Post-surgical instructions:

Sutures were removed two weeks after surgery.The patients were instructed to abstain from mechanical oral hygiene procedures in the surgical area for 4 weeks. 0.2% chlorhexidine mouth rinse was used to support plaque control. professional plaque removal was performed for every 2 weeks, up to the sixth week.Each patient was to report thereafter for a monthly maintenance regime for six months.Each recall visit included reinforcement of oral hygiene procedure, supragingival plaque and calculus removal if necessary.

Statistical Analysis

The data was analyzed statistically using SPSS software version.22to find the mean, standard deviation, and test of significance. Intra group analysis was done bypaired t tests and the Inter group analysis was done with Levene's test for equality of variances. p value of ($p \leq 0.05$) was considered statistically significant.

Results

The healing process went without complications. The flap closure was completeand no flap dehiscence was observed,thus providing primary healing process. Table 1 shows the descriptive details of the control and test subjects at baseline,12 months, and 36 months. Pre-operative Clinical Parameters were similar for the both the SFA+PRF+HA -βTCP (test) group and SFA+PRF (control) group.

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Table 1. Descriptive Statistics

Clinical Parameters	N	Baseline		12 Months		36 Months	
		Control	Test	Control	Test	Control	Test
FMPS%	15	17.2 ± 1.32	16.4 ± 1.96	12.03±1.24	12.3±1.48	11.3±1.11	11.5±1.04
FMBS%	15	18±1.06	18.3±1.28	12.4±1.31	12.3±1.13	11.7±1.16	11.6±0.98
PPD (mm)	15	6.8±0.77	7.06±1.39	2.6±0.51	2.53±0.52	2.6±0.51	2.8±0.56
RCAL (mm)	15	10.2±0.82	10.5±1.38	6.4±3.9	6.06±0.78	6.4±0.46	6.4±0.53
GM (mm)	15	3.43±0.68	3.4±0.58	3.8±0.61	3.5±0.61	3.5±0.76	3.3±0.37
RVG (mm)	15	3.7±0.94	3.7±0.92	2.23±0.66**	1.4±0.47**	1.38±0.62	0.92±0.39

Post -surgical measurements at 12 months and 36 months revealed a significant reduction in the mean FMPS%, FMBS%, & PPD within the groups **(p≤0.0001),** but they did not reveal any statisticalsignificance when compared between the test and the control groups at various time points. (Table 2 &3)

Table 2. Intra group assessment of Clinical and Radiographic parameters

Parameters	Groups	Baseline - 12 Months		12 Months - 36 Months		Baseline - 36 Months	
		Mean ± SD	P value	Mean ± SD	P value	Mean ± SD	P value
FMPS	Control	17.2 ± 1.32	0.0001	12.03±1.24	0.002	17.2 ± 1.32	0.0001
		12.03±1.24		11.3±1.11		11.3±1.11	
	Test	16.4 ± 1.96	0.0001	12.3±1.48	0.005	16.4 ± 1.96	0.0001
		12.3±1.48		11.5±1.04		11.5±1.04	
FMBS	Control	18±1.06	0.0001	12.4±1.31	0.001	18±1.06	0.0001
		12.4±1.31		11.7±1.16		11.7±1.16	
	Test	18.3±1.28	0.0001	12.3±1.13	0.001	18.3±1.28	0.0001
		12.3±1.13		11.6±0.98		11.6±0.98	
PPD	Control	6.8±0.77	0.0001	2.6±0.51	1.000	6.8±0.77	0.0001
		2.6±0.51		2.6±0.51		2.6±0.51	
	Test	7.06±1.39	0.0001	2.53±0.52	0.057	7.06±1.39	0.0001
		2.53±0.52		2.8±0.56		2.8±0.56	
GM	Control	3.43±0.68	0.048	3.8±0.61	0.060	3.43±0.68	0.788
		3.8±0.61		3.5±0.76		3.5±0.76	
	Test	3.4±0.58	0.784	3.5±0.61	0.094	3.4±0.58	0.388
		3.5±0.61		3.3±0.37		3.3±0.37	
RCAL	Control	10.2±0.82	0.0001	6.4±3.9	0.085	10.2±0.82	0.0001
		6.4±3.9		6.4±0.46		6.4±0.46	
	Test	10.5±1.38	0.0001	6.06±0.78	0.685	10.5±1.38	0.0001
		6.06±0.78		6.4±0.53		6.4±0.53	
RVG	Control	3.7±0.94	0.0001	2.23±0.66	0.0001	3.7±0.94	0.0001
		2.23±0.66		1.38±0.62		1.38±0.62	

Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 6, 2021, Pages. 10430 - 10440
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	Test	3.7±0.92	0.0001	1.4±0.47	0.0001	2.78±0.87**	0.0001
		1.4±0.47		0.92±0.39		0.92±0.39	

Table 3. Inter group assessment of Clinical and Radiographic parameters

Parameters	N	Groups	Baseline		12 Months		36 Months	
			Mean ± SD	P value	Mean ± SD	P value	Mean ± SD	P value
FMPS	15	Control	17.2 ± 1.32	0.2	12.03±1.24	0.598	11.3±1.11	0.506
	15	Test	16.4 ± 1.96		12.3±1.48		11.5±1.04	
FMBS	15	Control	18±1.06	0.403	12.4±1.31	0.825	11.7±1.16	0.674
	15	Test	18.3±1.28		12.3±1.13		11.6±0.98	
PPD	15	Control	6.8±0.77	0.521	2.6±0.51	0.724	2.6±0.51	0.240
	15	Test	7.06±1.39		2.53±0.52		2.8±0.56	
RCAL	15	Control	10.2±0.82	0.476	6.4±3.9	0.087	6.03±0.69	0.678
	15	Test	10.5±1.38		6.06±0.78		6.13±0.61	
GM	15	Control	3.43±0.68	0.886	3.8±0.61	0.147	3.5±0.76	0.365
	15	Test	3.4±0.58		3.5±0.61		3.3±0.37	
RVG	15	Control	3.7±0.94	0.974	2.23±0.66	0.0001	1.38±0.62	0.023
	15	Test	3.7±0.92		1.4±0.47		0.92±0.39	

Both test and control treatments resulted in a statistically significant gain in the mean Relative Clinical Attachment Levels (R-CAL) of 4.467±1.2mm 3.767±.942mm at six months **(p≤0.0001).** Differences in R-CAL gain between the two treatment modalities were comparable between both the groups at all time points. (Table 2 &3)

The position of the gingival margin (GM) had a continual gradual apical shift (gingival recession) till 12 months which averaged to -0.433±.77 mm with a mild statistical significance of **(p≤ 0.048)** in the control group. The test group initially had a coronal shift in the 12monthstime point and then shifted apically later and became comparable at 36 months between the control and the test group with no statistical significance(p≤0.784). (Table 2 &3)

Additionally RVG values revealed a statistically significant bone fill **(p≤0.0001)** in both, test group (2.30 ± 0.79mm) and the control group (1.47±0.49mm) at 12 months, this radiographic bone fill was greater and statistically significant in the test group when compared with the control at the 12months . (p≤0.0001). (Table 2 &3)

when the difference in the bone fill was measured at 36 months between test (2.30 ±0.58 mm) and control (2.78 ±0.87mm) the difference was almost comparable between the groups at this time point with minimal significance.(p≤0.023). (Table 2 &3)

Discussion

The clinical procedure of choice for intrabony defects is the guided tissue regeneration (GTR) approach with a barrier membrane aiming clot and periodontal wound stability with the bone graft filling the defect.This study sought to assess the ability of a third tenet that has become louder over the last decade.Clinical studies arising from

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Received 25 April 2021; Accepted 08 May 2021.

Cortellini and also from Trombelli,have shown that their MIST & SFA technique have been able to generate significant periodontal tissue regeneration.[14,15,16,17,18,19]
Present study was a comparative evaluation of SFA+PRF & SFA+PRF+β-TCP in the treatment of the intrabonydefects present in the 30 patients. Periodic oral prophylaxis was performed so as to avoid formation of plaque, calculus deposits & debris on the surgicalsite as they could hamper the final out come.The highly significant gingival and plaque scores and bleeding scores in both the groups are in agreement with the previous studies demonstrating that oral hygiene and also the potential effects of PRF in the uneventfullhealing process.The most important surrogate marker of periodontal disease is that of probing pocket depth and is one of the primary objectives of the study. In our study there was a marked reduction in the Probing pocket depth with both test and control groups from baseline to various time points which were also a significant find in other studies by Trombelli et al[14]and Hanna et al[16]

The primary reason for reduction in depth after treatment can be attributed to the minimally accessed surgical technique (Single Flap Approach). The design of this surgical approach allows both access to root surface instrumentation and minimization of flap elevation through the elevation of the buccal flap alone. This further enhances wound stability during early wound healing and prevents the collapse of the papilla into the defect: at the end of the procedure, the buccal flap is repositioned and sutured to the interdental supracrestal soft tissues, still anchored with their fibres to the root cement. The improved stability of the soft tissues could play a positive role in increasing the stability of the blood clot, a key factor in regenerative therapy.[1]In addition, the potential prevention of the interdental soft tissue collapse could preserve more space for the regeneration to occur. The PRF also has been shown in earlier studies to promote the proliferation of periodontal ligament fibroblasts as well as gingival fibroblasts. Recently, studies have demonstrated that PRF has a very significant slow sustained release of growth factors such as PDGF and (TGF)-β for atleast 1 week and upto 28 days and this enhancement of the optimal healing period by PRF, ofthe periodontal wound creates greater opportunity for the regeneration of the lost periodontal tissues.[18]The PPD reduction although had progressive rate of reduction in both the groups, remained similar between the two groups suggesting that both treatment procedures ensure conditions for primary intention healing supporting adequate wound stability allowing uneventful tissue formation and maturation.[20,21]Most importantly the highly significant decrease in the probing pocket depth scores at all the evaluation time points, can occur due to combination of gain in clinical attachment as well as post treatment gingival margin shift to apical position in our study, that was seen in the control and test sites all of which showed continual progressive gain.[22,23]

The gain in clinical attachment level also reflected in the positive outcome to the position of the gingiva. The position of the gingival margin had a continual apical shift in the control group and was statistically significant when compared at baseline to12 months. ($p \leq 0.05$). On the other hand although the test group had an initial coronal shift in the position of the gingival margin at the time point of month, this level shifted apically in the 36thmonth and showed no statistical difference. These positions of the gingival margins at the surgical sites when compared between the test and the control groups were similar at all evaluation time points.

These clinical outcomes were further reflected in the gain in bone at the crest of the interproximal region,that was assessed through individually standardized digital radiographs. Both the control and the test groups had a significant gain in the crestal bone levels with a very highstatistical significance ($p<0.0001$).The Intergroup comparison further indicated the improvement to the bone levels, which showed a highly significant, formation of alveolar bone in the test group(2.23±.66mm),(1.38±0.62), in comparison to the controls(1.4±.47mm), (0.92±0.39) at 12 and 36 month time points ($p<0.0001$).In the standardized radiographs, 12 months after treatment with the composite graft, the filling of radiographic defects with bone -like radio opaque tissue, was observed and indistinguishable from native bone. The gain in the bone levels in the test group could have been due to the application of HA -βTCP as a bone replacement graft which has demonstrated its osseointegrative properties in other studies. It is accepted that the suitable biomaterials used for bone regeneration should be resorbable and gradually replaced by the newly formed bone. β -TCP has a low degradation rate and takes a longer period to be replaced by new bone tissue. Previous

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histologic studieshave shown that β –TCP particles are resorbed in 12 -24 months and replaced with the newly generated bone in 36 months. Wiltfang et al.[24] compared the graft materials with PRP in pigs and they observed β –TCP particles remaining in the bone defects at 12th week. At the end of the 12th week of the study by Yilmaz et al[25], β-TCP particles were observed histologically in the cavities that contained β –TCP alone, but no β -TCP particles were observed in the bone marrow when β -TCP and PRF has been used in combination. As a result, it can be stated that PRF increases the transformation of β -TCP particles into bone. Adding PRF to β–TCP was observed to significantly reduce the time required to promote graft consolidation, maturation, and improved trabecular bone density. Nery et al.[26] studied the tissue response to BCP ceramic with different ratios of HA/βTCP in periodontal osseous defects. They found that among the seven "active" treatment groups, two (65/35 and 85/15) had significantly higher gain in probing attachment levels than those in three group (50/50, 100/0, and 0/100) ($p< 0.05$). Histologically, higher HA ratio (but not 100% HA) showed accelerated new bone formation and new attachment levels. Based on histological results, the 85 HA/15 β-TCP ratio appears to demonstrate greater gain in attachment level and bone regeneration in the treatment of periodontal osseous defects. However human histologic studies indicate that dense HA and β-TCP does not induce new attachment or bone formation, that pocket reduction is primarily through fibrous encapsulation of the graft particles in the intraosseous defect, and that pocket closure is through long junctional epithelium and connective tissue adhesion. In our study accessed with SFA, when the PRF+HA/β-TCP combination group was compared with the PRF group, more radiographic bone fill was observed. It is thought that PRF accelerates the healing effect by keeping the particles of HA/β-TCP together via its adhesive property and adapting them tightly to the walls of the cavity. Although different studies have shown that there is complete biodegradation and new bone formation only histologic analysis can provide evidence on stimulated new bone formation.

Although different studies[27,28]have shown that there is complete biodegradation and new bone formation only histologic analysis can provide evidence on stimulated new bone formation.The different distribution of patients observed in control and test groups according to defect morphology may represent a potential source of bias since it has been reported that defect morphology is associated with varying regenerative potential.[29] The test group had 66.6% of two walls and 33.3% of three walls and there is also a possibility that variations in this supracrestal and osseous defect characteristics might have influenced our treatment outcome. The presence of a three - wall component conceptually is one determinant for the outcome of a reconstructive procedure because it may safeguard the stability of the fibrin clot and positively influence the osteogenic potential of the site. [30]

Conclusion

To the best of our knowledge, clinical data demonstrating an improved prognosis for three -wall versus one-or two-wall intraosseous defects after surgical debridement alone clearly segregating the depth of the intrabonycomponents are not available for comparison. However, when intraosseous defects are treated using a graft biomaterial or barrier membrane, defect configuration does not seem to significantly affect the amount of CAL gain. Despite these limitations, this study has proved in principle that PlateletRich Fibrin when grafted to a periodontal defect, which is approached through a minimal access periodontal surgical technique, has a high potential to favorably modulate the healing periodontal wound with or without an additional graft material.

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