Original Article

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Dentistry Section

Efficacy of Nanocrystalline Calcium Sulphate Bone Graft (NanoGen®) and Platelet Rich Fibrin in the Treatment of Periodontal Intrabony Defects: A Split Mouth Randomised Clinical Study

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ABSTRACT

Introduction: Periodontal disease leads to the loss of supporting structures of the tooth. Recent years have witnessed the evolution of many regenerating materials that have shown to be effective in regenerating the loss structures.

Aim: To evaluate and compare clinically and radiographically the efficacy of Platelet Rich Fibrin (PRF) and Nanocrystalline Calcium Sulphate Bone Graft (NanoGen®) in the treatment of intrabony defects.

Materials and Methods: A split mouth randomised clinical study was conducted in the Department of Periodontology, DAPM RV Dental College, Bangalore from November 2018 to May 2020. In this study, 30 surgical sites were selected from 15 chronic periodontitis patients American Academy of Periodontology (AAP, 1999) of age between 35-65 years and with interproximal probing depth ≥5 mm following phase I therapy and radiographic evidence of intrabony defects ≥3 mm deep. They were divided into two groups: Group I (n=15) received open flap debridement with nanocrystalline Calcium Sulphate (nCS) and Group II (n=15) open flap debridement with PRF. Clinical parameters assessed were Gingival Index (GI), Plaque Index (PI), Gingival Recession (GR), Probing Pocket Depth (PPD) and Clinical Attachment Level (CAL). Intragroup was compared using Repeated Measures of Analysis of Variance (ANOVA) followed by Bonferroni's Post hoc test and intergroup was compared using Independent student's t-test.

Results: Total 15 patients were selected in each group of which 10 were male and 5 were female patients. Mean age of the patients was 39.3 years. There was significant reduction in GI (p-value=0.04), PPD (p-value=0.04) and gain in CAL (p-value=0.04) in group I. The mean difference in CAL at six months was also statistically significant in group II (p-value=0.01). The mean difference of PI was not statistically significant between baseline to three months and baseline to six months in both groups. GR increased from baseline to three months and remained same at six months (p-value for group I and group II=0.36) in both groups. On intergroup comparison, group I (nCS) showed better improvement in clinical parameters like PPD (p-value=0.01), CAL (p-value=0.01) and BF (p-value=0.002) at all time intervals compared to group II (PRF).

Conclusion: There was improvement in all clinical parameters except GR in both groups. So both can be used as regenerative materials. But based on this study nanocrystalline calcium sulphate bone graft can be preferred over PRF as a regenerative material.

Keywords: Bone fill, Bone grafting, Periodontal disease, Regeneration

INTRODUCTION

Periodontal diseases include a wide range of inflammatory conditions that affect the tooth supporting tissues and can lead to loss of tooth and contribute to systemic inflammation [1]. The characteristics features of periodontal disease include gingival inflammation, periodontal pocket formation, and loss of connective tissue attachment and alveolar bone around the affected teeth [2]. Once the inflammatory aspect of the disease has been controlled, then the periodontal therapy is aimed at the regeneration of the destroyed tissues [3].

At present different types of bone grafts are used. According to their origin they have been classified as: autografts (obtained from the same patient), allograft (the same species but a different individual), xenograft (different species) and alloplast (synthetic graft). Depending on their action on bone, they were attributed with osteogenic, osteoinductive or osteoconductive capabilities [4].

Calcium sulphate has been used in the field of dentistry for more than 30 years. The use of Plaster of Paris to fill bone defects in dentistry was introduced by Bahn [5]. Calcium sulfate is gaining attention because of its biocompatibility and handling characteristics, porosity and different rates of dissolution, chemical and physical similarity to bone minerals. They act as resorbable osteoconductive scaffolds that provide osteogenesis and prevent tissue invasion, thus act as space filler [6].

Degradation of calcium sulphate can be explained by two mechanisms. Initially there is release of sulphur and calcium ions in the biological environment that results in the formation of carbonate and stimulation of calcium ion in cellular activity. The second mechanism involves precipitation of calcium phosphate, that will lead to a transient fall in pH. This results in demineralization of existing bone leading to exposure of bioactive molecules. This leads to release of growth factors like bone morphogenetic proteins and transforming growth factor, which stimulates the growth of bone [7].

Recently nanocrystalline forms of bone graft with smaller particle size is gaining attention, which have advantages of sustain release, and slower rate of resorption as compared to normal size particles [8]. NanoGen® is a recent product which contain nanocrystalline calcium sulphate with particle size of 200 to 900 nanometers. When mixed with saline, putty consistency is obtained, making the material easy to handle and moldable. After placement, it undergoes controlled

degradation within three to four months. This inturn leads to the deposition of calcium phosphate that stimulates the formation of bone [8].

Regenerative potential of the platelet was introduced in the 70's as they are rich source of growth factors like Platelet Derived Growth Factor (PDGF), Insulin like Growth Factor (IGF), Transforming Growth Factor-β (TGFβ), which regulates the main events in tissue regeneration [9,10]. Choukroun J et al., was the first to develop Platelet Rich Factor (PRF) in France for specific use in oral and maxillofacial surgery [11]. It have several advantages like ease in preparation and not requiring chemical manipulation of the blood, which makes it strictly an autologous preparation [12].

There are paucity of studies that compares the effectiveness of PRF and nanocrystalline calcium sulphate in intrabony defects. Hence, this study aims to evaluate and compare the treatment of intrabony defects by using nanocrystalline calcium sulphate and PRF.

MATERIALS AND METHODS

A split mouth randomised clinical study was conducted in the Department of Periodontology DAPM RV Dental College, Bangalore to evaluate and compare the efficacy of PRF and nanocrystalline calcium sulphate bone graft (NanoGen®) in the treatment of intrabony defects. The study population was selected from the subjects visiting the Outpatient Department of Periodontics from November 2018 to May 2020. The Ethical clearance for the study was obtained from the Ethical Committee and review board of the institution (285/VOL-2/2018). The participants were explained about the study and a written consent was obtained from each of the participants.

Inclusion criteria: Patients diagnosed with chronic periodontitis (AAP, 1999) and aged between 35 to 65 years of either gender [13]. Each patient should have atleast two sites with interproximal probing depth ≥5 mm following phase I therapy and also the sites exhibiting clinical evidence (patients maintaining good oral hygiene, gingival index score of less than 2.1 after two weeks of phase I therapy) and radiographic evidence of intrabony defects ≥3 mm deep [14,15] were included in the study.

Exclusion criteria: Subjects who have received periodontal flap/regenerative therapy within the past one year, pregnant and lactating patients, patients with uncontrolled diabetes and immuno-compromised patients, patients who were under antibiotics analgesics, steroids for the past three months, smokers and patients who demonstrated poor oral hygiene maintenance, with a gingival index score ≥2.1 after two weeks of phase I therapy were excluded from the study.

Sample size calculation: The sample size was estimated using the G*Power software v. 3.1.9.2). Considering the effect size to be measured (d) at 80% for one tailed hypothesis, power of the study at 80% and the margin of the error at 10%, the total sample size needed was 30 [16].

Thirty surgical sites were selected and divided into two groups [Table/Fig-1]:

Group I (n=15)- Those treated with with open flap debridement along with nanocrystalline calcium sulphate

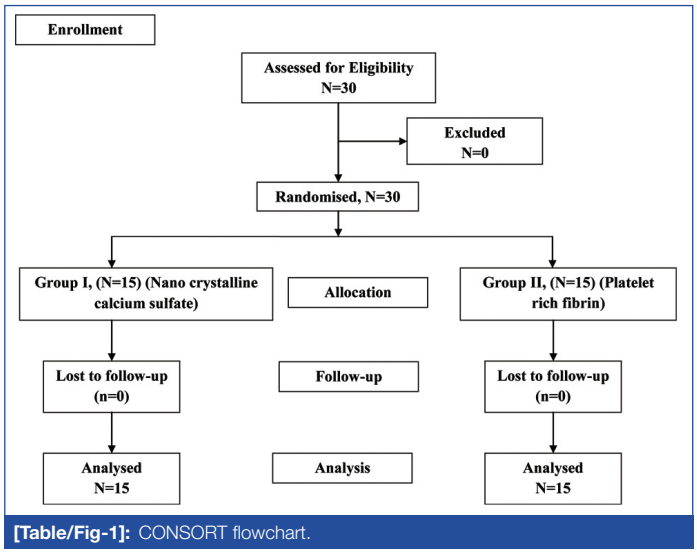
Group II (n=15)- Those treated open flap debridement along with PRF

Study Procedure

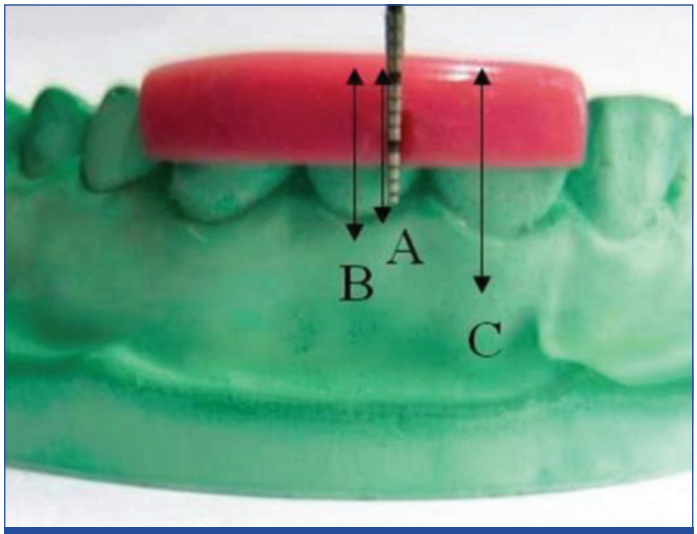
1. Presurgical procedures: Case history was recorded, clinical photographs were taken and study casts were made for all the patients. Routine laboratory investigations, complete haemogram and random blood sugar were done. Scaling and root planning was performed using hand and ultrasonic instruments. Trauma from occlusion, if present, was relieved. Adjunctive chemical plaque control in the form of chlorhexidine mouthwash 0.2% twice daily was advised. Patients were re-evaluated two weeks after phase I therapy.

Oral hygiene status was assessed using Plaque Index {Silness and Loe (1964)} and Gingival index {Loe and Silness (1963)} [17].

Customised acrylic stent was made for each patient to record PPD, CAL and GR using University of North Carolina (UNC) 15 probe [Table/Fig-2].



[Table/Fig-1]: CONSORT flowchart.



[Table/Fig-2]: Measurement of clinical parameters; a) Stent to cementoenamel junction; b) Stent to gingival margin; c) Stent to deepest probing depth at test sites.

For that an alginate impression was made using metallic tray for each patient. The models were made using dental stone. Customised acrylic stents were prepared on the model for each patient using auto polymerizing acrylic resin. Vertical guiding grooves were made on the stent at the defect site to guide probe penetration with the same position and angulation, thereby providing a well-defined and reproducible clinical measurement at each site for examination. All parameters were assessed at baseline (during surgery) and after three months and six months postsurgery.

Measurements will be recorded from:

Stent to cementoenamel junction-A

Stent to gingival margin-B

Stent to deepest probing depth at test sites-C

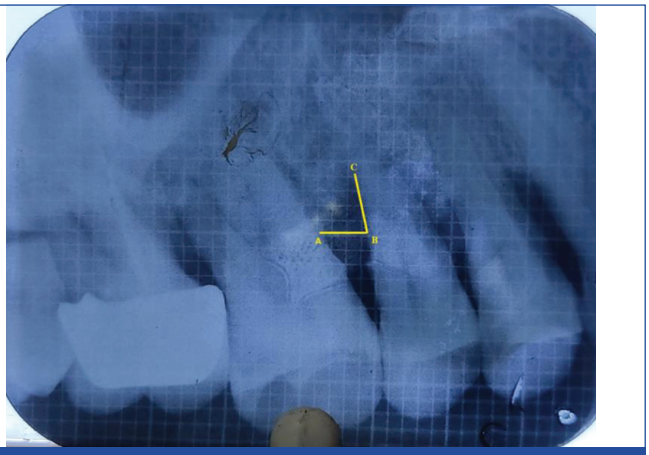
2. Calculation of the parameters [18]:

Probing Pocket Depth (PPD)=Stent to deepest probing depth at test sites (C)-Stent to gingival margin (B)

Clinical Attachment Level (CAL)=Stent to deepest probing depth at test sites (C)-Stent to Cementoenamel Junction (A)

Gingival recession=Stent to gingival margin (B)-Stent to Cementoenamel junction (A)

Intraoral periapical radiographs were taken with radiographic grid using long cone paralleling technique at baseline (during surgery) and after three months and six months postsurgery to assess the Depth of the Defect (DOD) and Bone Fill (BF) [Table/Fig-3].



[Table/Fig-3]: Assessment of depth of defect.

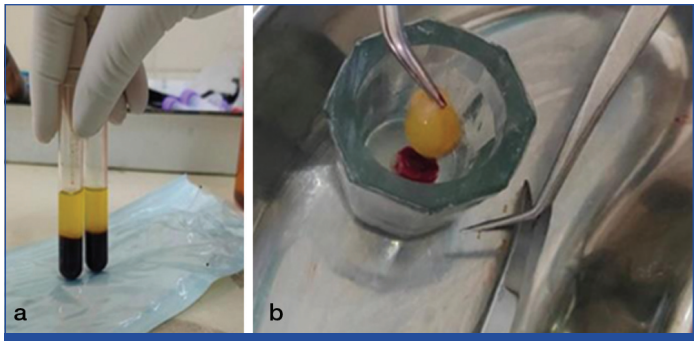
The depth of the bone defect was assessed to the closest 0.5 mm on the intraoral periapical radiograph. A horizontal line was drawn projecting from the point on the bone crest designated as 'A'. The horizontal line was drawn perpendicular to the long axis of the root surface of the tooth associated with the vertical defect and the point of contact of the horizontal line with the root surface was designated as 'B'. A vertical line was then drawn from 'B' to the most coronal level along the root surface where the periodontal ligament space was considered to have a normal width; the point was designated as 'C'. The vertical dimension between 'B' and 'C' was measured to assess the bone level and bone fill was calculated by taking the difference between baseline radiograph and 6 months radiograph.

3. Surgical protocol: Following administration of LA (2% lignocaine hydrochloride with 1 in 80,000 adrenaline) acrylic stent was placed and PPD, CAL and GR was recorded to the nearest millimeter with the help of a University of North Carolina (UNC) 15 probe (size, site and type of defect was different for different patient). After that buccal and lingual or palatal crevicular incisions were made using a no. 15 sterile surgical blade. A full thickness mucoperiosteal flap was reflected with molt no. 9 periosteal elevator. Careful defect debridement and root planing was done using ultrasonic instruments and area specific curettes (Gracey curettes, Hu- Friedy, Chicago, IL, USA), following which the surgical site was completely irrigated with povidone iodine. Before the placement of the graft or PRF, a 3-0 non resorbable braided silk suture was passed through the buccal and palatal or lingual papillae and the suture was left loose. This was done in order to prevent removal of the graft particles/ PRF by the passage of the needle and suture material.

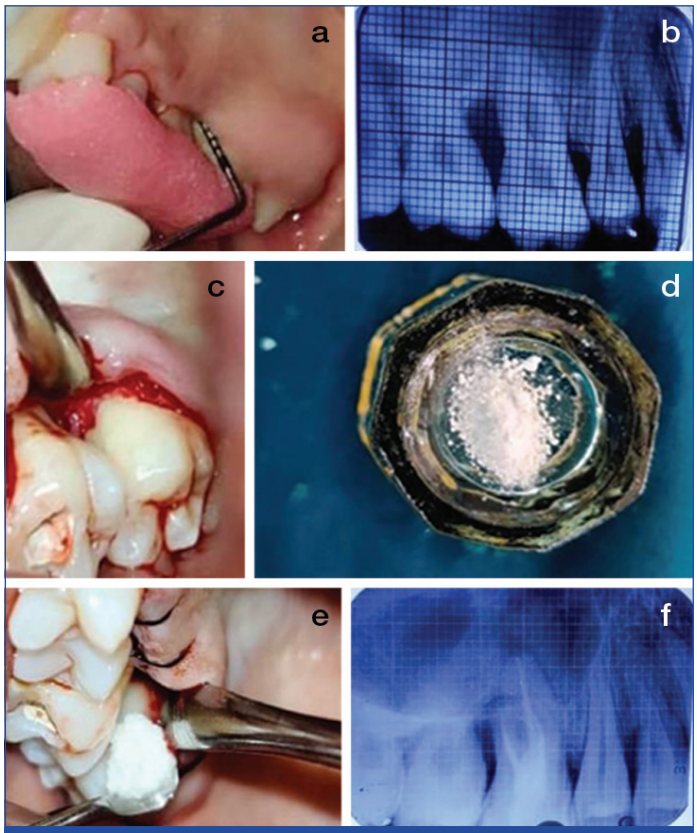
4. Preparation of PRF: The PRF was prepared using Choukroun J et al., protocol [11]. About 5 mL of patient's intravenous blood was drawn and centrifuged at 3000 rpm for 10 minutes in a table top centrifuge. A top layer of acellular plasma (Platelet Poor Plasma-PPP) and bottom layer of red corpuscles was formed [Table/Fig-4a]. Between these two layers, a structured fibrin clot was found that was removed along with a small layer Red Blood Cell (RBC) present at the bottom using tweezer and scissors and then transferred to a sterile dappen dish [Table/Fig-4b].

Group I patients received nanocrystalline calcium sulphate bone graft [NanoGen®] while group II patients received PRF. The graft material was mixed with saline and the defect was filled till the rim of the defect [Table/Fig-5a-f]. The suturing was then completed and non eugenol periodontal dressing (Coe pack™, GC America Inc., Chicago, IL, USA) was placed for one week.

5. Postsurgical care: Suitable antibiotics and analgesics were prescribed [tablet ciprofloxacin (500 mg)+tinidazole (600 mg), two



[Table/Fig-4]: a) PRF preparation; b) PRF transferred to dappen dish.



[Table/Fig-5]: a) PPD measurement at Baseline irt 17; b) Baseline Intraoral Periapical radiograph (IOPA); c) Flap reflection and debridement; d) NanoGen Bone graft; e) Bone graft placement; f) IOPA after 6 months.

times daily for five days and tablet aceclofenac (100 mg)+paracetamol/ acetaminophen (325 mg)+serratopeptidase (15 mg) twice daily for three days]. Patients were advised to rinse with chlorhexidine digluconate (0.2%) twice a day for two weeks following surgery and advised not to brush the surgical site for 7-10 days. Periodontal dressing and sutures were removed one week after surgery. Patients were instructed to use soft toothbrush and not to floss and use any interdental aids in the area for four weeks. Each patient was re-instructed for proper oral hygiene measures at every recall review.

STATISTICAL ANALYSIS

Statistical Package for Social Sciences (SPSS) for Windows Version 22.0 Released 2013. Armonk, NY: IBM Corp was used to perform statistical analyses. Independent Student's t-test was used to compare the mean clinical and radiological parameters at baseline and postintervention time intervals between 2 groups. Repeated measures of ANOVA followed by Bonferroni Post hoc analysis were used to compare clinical and radiological parameters between time groups in each study group. The level of significance was set at $p<0.05$.

RESULTS

Total 15 patients were selected in each group, of which 10 were male patients and 5 were female patients. Of the selected 15 patients, mean age of the patients were 39.3 years.

Plaque Index (PI)

On intragroup comparison mean plaque index scores for group I and II at baseline, three months and six months were 1.49±0.18, 1.13±0.18, 1.11±0.20 and 1.49±0.18, 1.14±0.19 and 1.14±0.23 respectively. The mean difference in PI was not statistically significant between baseline and three month and baseline and six months in both groups (p-value for group I=0.91 and group II=1.00). On intergroup comparison the mean difference in the values between two groups showed no significant difference at any time interval (p>0.05) [Table/Fig-6].

Time	Groups	Mean	SD	Mean difference	p-value
Baseline	Group I	1.49	0.18	0.00	1.00
	Group II	1.49	0.18		
3 months	Group I	1.13	0.18	0.01	0.87
	Group II	1.14	0.19		
6 months	Group I	1.11	0.20	0.03	0.73
	Group II	1.14	0.23		

[Table/Fig-6]: Plaque index- Intergroup comparison using using Independent Student's t-test.
*Statistically significant

Gingival Index (GI)

On intragroup comparison mean gingival index scores for group I and II at baseline, three months and six months were 1.62±0.21, 1.24±0.17 and 1.20±0.15 and 1.62±0.21, 1.23±0.17, 1.22±0.24 respectively. The mean difference in GI was statistically significant between baseline to three months to baseline and six months in group I (p-value for group I=0.04) and not significant in group II. On intergroup comparison, the mean difference in the values between two groups showed no significant difference at any time interval (p>0.05) [Table/Fig-7].

Time	Groups	N	Mean	SD	Mean difference	p-value
Baseline	Group I	15	1.62	0.21	0.00	1.00
	Group II	15	1.62	0.21		
3 months	Group I	15	1.24	0.17	0.00	0.95
	Group II	15	1.23	0.17		
6 months	Group I	15	1.20	0.15	-0.03	0.73
	Group II	15	1.22	0.24		

[Table/Fig-7]: Intergroup comparison of mean values of GI at baseline, 3 months, 6 months period between 2 groups using independent student t-test.
*Statistically significant

Probing Pocket Depth (PPD)

On intragroup comparison mean PPD for group I and II at baseline, three months and six months were 9.4±1.77, 5.13±0.83, 4.67±0.90 and 9.33±2.19, 5.93±1.22, 5.87±1.46 respectively. The mean difference in PPD was statistically significant between baseline to three months and baseline to six months in group I (p-value for group I=0.04). On intergroup comparison the mean difference in the values between two groups at baseline, three months and six months were -0.07, -0.80 and -1.20, respectively [Table/Fig-8]. Group I showed statistically significant decrease in PPD at three months (p-value=0.04) and six months when compared to group II (p-value=0.01).

Time	Groups	Mean	SD	Mean difference	p-value
Baseline	Group I	9.40	1.77	-0.07	0.93
	Group II	9.33	2.19		
3 months	Group I	5.13	0.83	-0.80	0.04*
	Group II	5.93	1.22		
6 months	Group I	4.67	0.90	-1.20	0.01*
	Group II	5.87	1.46		

[Table/Fig-8]: Probing Pocket Depth (PPD)- Intergroup comparison using independent Student's t-test.
*Statistically significant

Gingival Recession (GR)

On intragroup comparison mean GR for group I and II at baseline, three months and six months were 1.00±0.00, 1.08±0.29, 1.17±0.39 and 1.00±0.00, 1.14±0.36, 1.14±0.36 respectively. Both groups showed increase in gingival recession from baseline to three months and remained the same at six months. The mean difference in gingival recession was not statistically significant between all the time intervals in both groups (p-value=0.36). On intergroup comparison the mean difference in the values between two groups at three months and six months were -0.06 and 0.03 respectively [Table/Fig-9]. The difference in the mean gingival recession was found to be non significant between all the time intervals (p-value=0.87).

Time	Groups	Mean	SD	Mean difference	p-value
Baseline	Group I	1.00	0.00	-	-
	Group II	1.00	0.00		
3 months	Group I	1.08	0.29	-0.06	0.65
	Group II	1.14	0.36		
6 months	Group I	1.17	0.39	0.03	0.87
	Group II	1.14	0.36		

[Table/Fig-9]: Gingival Recession (GR)- Intergroup comparison using independent student t-test.
*Statistically significant

Clinical Attachment Level (CAL)

On intragroup comparison mean CAL for group I and II at baseline, 3 months and 6 months were 9.87±1.77, 5.93±1.22, 5.47±1.19 and 9.93±1.77, 6.93±1.49, 6.87±1.69 respectively. The mean difference in CAL was statistically significant between baseline to three months and baseline to six months in both groups (p-value for group I=0.04 and group II=0.01). On intergroup comparison the mean difference in the values between two groups at baseline, three months and six months were -0.06, -1.00 and -1.40 respectively [Table/Fig-10]. The differences were statistically significant at three months and six months with group I showing a greater gain in CAL (p-value=0.01).

Time	Groups	Mean	SD	Mean difference	p-value
Baseline	Group I	9.87	1.77	-0.06	0.93
	Group II	9.93	2.15		
3 months	Group I	5.93	1.22	-1.00	0.04*
	Group II	6.93	1.49		
6 months	Group I	5.47	1.19	-1.40	0.01*
	Group II	6.87	1.69		

[Table/Fig-10]: Clinical Attachment Level (CAL)- Intergroup comparison using independent student t-test.
*Statistically significant

Depth of Defect (DOD) and Bone Fill (BF)

On intragroup comparison mean DOD for group I and II at baseline, three months and six months were 7.20±1.15, 4.60±1.35, 4.07±1.79 and 6.80±1.15, 5.20±2.08, 5.53±1.96 respectively. The mean difference in DOD was not statistically significant between baseline to three months, baseline to six months in both groups (p-value for group I=0.45 and group II=0.17). On Intergroup comparison the mean difference in the values between two groups at baseline, three months and six months were 0.40, -0.60 and -1.46 respectively which was statistically significant at six months [Table/Fig-11,12]. At six months mean depth of defect was significantly lesser in test group as compared to control group and the difference was statistically significant (p-value=0.04.)

At six month's period the mean amount of bone fill in group I was 3.13 and group II was 1.20. Bone fill in group I was significantly higher as compared to group II and the difference was statistically significant (p-value=0.002).

Time	Groups	Mean	SD	Mean difference	p-value
Baseline	Group I	7.20	1.15	0.40	0.35
	Group II	6.80	1.15		
3 months	Group I	4.60	1.35	-0.60	0.36
	Group II	5.20	2.08		
6 months	Group I	4.07	1.79	-1.46	0.04*
	Group II	5.53	1.96		

[Table/Fig-11]: Depth of defect (DOD)- Intergroup comparison using independent student's t-test.
*Statistically significant

Time	Groups	Mean	SD	Mean difference	p-value
6 months	Group I	3.13	1.69	1.93	0.002*
	Group II	1.20	1.42		

[Table/Fig-12]: Comparison of mean bone fill at 6 month's period between two groups using independent student's t-test.
*Statistically significant

DISCUSSION

The result of present study showed that both nanocrystalline calcium sulphate and PRF resulted in improvement in clinical parameters. The main aim of periodontal regeneration is the formation of new tooth-supporting tissues including cementum, PDL, and alveolar bone on a previously diseased root surface. Many materials that are available in the market have shown promising results. Among this newer regenerative materials are PepGen P-15, GEM 21S®, hydrogels, nanofibrous scaffolds, nano/microspheres, and multiphase scaffolds [19-21].

Calcium sulphate hemihydrate is completely synthetic, biocompatible, biodegradable and a highly osteoconductive material and is the only bone graft that possesses haemostatic, angiogenic and barrier membrane properties. It is a potent vehicle for delivery of growth factor and can be used along with other bone graft materials [22]. Strocchi R et al., (2002) demonstrated the ingrowth of blood vessels into the defects loaded with calcium sulfate than those with autograft [23]. Several drugs like Tobramycin (Beardmore AA et al., in 2005), Simvastatin (Nyan M et al., in 2007) and Daptomycin (Webb ND et al., in 2008) have been delivered locally through calcium sulfate [24-26].

But calcium sulphate dissolves rapidly at a rate of 1 mm per week. At times, its degradation outpaces the rate of new bone growth into the defect. To overcome this nanocrystalline calcium sulfate particles based bone graft was developed [27]. Particles of nanocrystalline calcium sulphate consist of densely packed grains of calcium sulfate in smaller particle size. These particles degrade in 12 to 14 weeks compared to standard calcium sulphate which degrade in four to six weeks. Hence nanocrystalline calcium sulphate has advantages like sustained release with slower rate of resorption as compared to medical grade calcium sulphate [8].

There are many natural materials used for periodontal regeneration. Among them PRF is one natural scaffold which showed promising results. PRF predominantly consists of a fibrin matrix rich in platelet and leukocyte, cytokines such as Interleukin-1 (IL-1), IL-4, and IL-6, and growth factors. Fibrin gels are formed in the final stage of the coagulation cascade in which fibrinogen molecules self assemble into a highly biocompatible three dimensional fiber network. The combination of fibrins and cytokines within PRF makes it a strong bio scaffold with an integrated reservoir of growth factors for tissue regeneration [28].

The PPD, CAL and GR were assessed using a UNC 15 probe positioned along a customised acrylic stent for providing a reproducible insertion axis for the probe. In a study by Watts T, probing depth and CEJ assessed by a constant force probe with and without stent. The result showed that stent to CEJ showed the maximum reproducibility (53%) of the simple measurements [29].

The depth of the intrabony defects was assessed with intraoral periapical radiographs using radiographic grid. Radiographic grid reduces the inaccuracy behind manual assessment of bone fill and the overestimation of BF, and it may be attributed to the enhanced sensitivity of the method [30]. Toback GA et al., conducted a study to assess the accuracy of radiographic grid and concluded that radiographic measurements using grid had significantly reduced the inaccuracy in comparison with conventional methods [30].

Plaque index and gingival index showed statistically significant decrease from baseline to three months and baseline to six months in group I and group II. No statistically significant difference was found among two groups. The improvement in gingival and plaque status may be due to good patient compliance. These results were in agreement with the studies conducted by Slot DE et al., and Stein JM et al, which showed improvement in gingival and plaque status in all the patients who maintained good oral hygiene [31,32].

The GR increased from baseline to three months and after that it remained constant at six months in both groups. The reason for increase in GR may be because of the gingival shrinkage during the healing period. These results were in agreement with the studies of Aichelmann-Reidy ME et al., [33]. Both groups showed statistically significant reduction in PPD at three months and six months. In intergroup comparison group I showed statistically significant reduction in pocket depth compared to group II at all time intervals. In group I patients this findings were in consistence with studies done by Das EC et al, Park YB et al., [34,35]. These studies showed that greater PD reduction seen in patients treated with calcium sulphate based bone graft materials. In group II patients the finding were in agreement with the previous studies done by Choukroun J et al., and Dohan Ehrenfest DM et al., [11,28].

Similarly, gain in CAL was observed in both groups. In intergroup comparison group I showed statistically significant gain in CAL compared to group II at all time intervals. Fibroblast growth factor is released in an active form from calcium sulphate and the release of the growth factor was directly proportional to the degradation rate of calcium sulphate, which facilitates migration of gingival fibroblasts and cell attachment and spreading, resulting in decrease of PPD and gain of CAL (Rosenblum SF et al.,) [36]. In group II patients, PD reduction and CAL gain may be related to the elevated concentrations of polypeptide growth factors, which might have enhanced soft tissue healing [28].

This study also assessed DOD and BF. Both groups showed statistically significant decrease in DOD and increase in BF. In intergroup comparison group I showed statistically significant reduction in DOD and increase in BF compared to group II at all time intervals. This improvement in parameters in both groups may be due to decrease in inflammation and regenerative potential of both nanocrystalline calcium sulphate bone graft and PRF [18]. Proper patient selection and patient compliance might be other reasons. In group I patients, these results were consistent with study done by Pandit N et al., Reddy MS et al., Couri CJ et al., and Paolantonio M et al., [18,37-39]. In group II patients the results are in accordance with study conducted by Patel GK et al., Chandradas ND et al., Pradeep AR et al., Suwondo CI and Galav S et al., [40-44].

The reason for group I showing better statistically significant improvement in parameters like PPD, CAL and BF, when compared to group II may be due to the haemostatic, angiogenic barrier membrane properties, slow resorption rate and sustained release of nanocrystalline calcium sulphate [23,45,46]. Also the surface area of nanocrystalline calcium sulphate was about 10 times greater than that of a conventional micron sized form which allows for greater absorption of growth factors, higher surface area for attachment of osseous cells and more efficient osteoconductivity [47]. But more multicenter randomised controlled clinical trial with large sample size will be required to confirm this result. Many similar studies in comparison to present study have been tabulated in [Table/Fig-13] [11,18,28,31-36, 38-44].

S. No.	Author's name and year	Place of study	Number of subjects	Materials used	Parameters compared	Conclusion
1.	Slot DE et al., 2020 [31]	Netherland	Systematic review (16 publications)	-		Self care by patients reduces gingival inflammation.
2.	Stein JM et al., 2010 [32]	Aachen	147	-	PI, GI, PD, CAL, BOP	Periodontal pathogens might be of particular value for the periodontal manifestation.
3.	Aichelmann-Reidy ME et al., 2004 [33]	University of Maryland Dental School	20	Calcium sulphate dehydrate	PD, GR and Attachment level	Improvement in all clinical parameters except GR.
4.	Das EC et al., 2019 [34]	Thiruvananthapuram, Kerala	-	Calcium sulfate-based bioactive bone cement (BioCaS)	Cytotoxicity and cytocompatibility	Potential candidate for the repair of periodontal defects.
5.	Park YB et al., 2011 [35]	Yonsei University College of Dentistry	-	Nanoparticles of Calcium Sulfate (nCS)	Cell viability/metabolic activity assays and alkaline phosphate assays	nCS can act as a scaffold to support osteoblastic cell activity.
6.	Choukroun J et al., 2006 [11]	Phoenix	9	FDBA with or without PRF	Healing time	FDBA and PRF leads to a reduction of healing time prior to implant placement.
7.	Dohan Ehrenfest DM et al., 2009 [28]	University of Gothenburg	4	PRF	Release of growth factors	PRF membrane sustains a very significant slow release of key growth factors.
8.	Rosenblum SF et al., 1993 [36]	New York	-	Plaster of Paris (POP)	Dissolution of PLP in various solutions	May serve as delivery vehicle for FGF to osseous tissues.
9.	Pandit N et al., 2015 [18]	DAV (C) Dental College and Hospital, Yamuna Nagar	16	Nanoge, Dentogen and Bonogen	PPD, CAL and Defect fill	NanoGen® and BoneGen can be considered for the treatment of infrabony periodontal defects.
10.	Couri CJ et al., 2002 [38]	Italy	13	Medical grade calcium sulfate hemihydrate	Keratinized gingival width, probing depth, and recession	Improvement in all clinical parameters.
11.	Paolantonio M et al., 2008 [39]	Mysore	51	Calcium Sulphate (CS)	PD, CAL, GR and defect bone level	CS have additional clinical benefits.
12.	Patel GK et al., 2017 [40]	Kanyakumari	13	PRF	PD, CAL and bone probing depth	Significant soft tissue healing and reduction in probing depth.
13.	Chandradas ND et al., 2016 [41]	Bangalore	36	PRF	PI, GI, PD, CAL and GR	PRF improves clinical and radiological parameters.
14.	Pradeep AR et al., 2017 [42]	Denpasar, Bali	62	PRF	PD, CAL, intrabony defect depth and % defect fill	Significant improvement of clinical parameters.
15.	Suwondo CI et al., 2018 [43]	Indonesia	10	A-PRF	PD, CAL and alveolar bone height	Enhanced periodontal tissue regeneration.
16.	Galav S et al., 2016 [44]	Jabalpur	20	PRF, Autogeneous bone graft	PPD, CAL surgical re-entry bone fill, and radiographic bone fill	Both ABG and PRF can be used predictably to reconstruct lost periodontal structures.
17.	Present study	Bangalore	15	NanoGen®, PRF	GI, PI, PPD, CAL, GR, DOD and BF	Both group showed improvement in all clinical parameters except GR.

[Table/Fig-13]: Comparison of similar studies in the literature [11,18,28,31-36,38-44].

Limitation(s)

The major limitation of the present study is its small sample size which is inadequate to evaluate the efficacy of graft material and also the intrabony defects included in our study differed in their dimension i.e., the width and depth. The treatment outcome is influenced by the differences in the dimensions of the defect.

CONCLUSION(S)

Treatment with both PRF and nanocrystalline calcium sulphate bone graft (NanoGen®) resulted in significant improvement in all parameters except gingival recession at all time intervals. In intergroup comparison, nanocrystalline calcium sulphate bone graft (NanoGen®) showed better improvement in parameters like PPD, CAL and BF at all time intervals. Hence, according to this study nanocrystalline calcium sulphate bone graft can be preferred over PRF as a regenerative material, especially in case of deep intrabony defects. But in situations which require more economical and easily available regenerative material, PRF can be preferred.

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