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Research Article

CLINICAL AND RADIOGRAPHIC EVALUATION OF NANOCRYSTALLINE HYDROXYAPATITE WITH ADVANCED PLATELET RICH FIBRIN (A-PRF) AND PLATELET RICH FIBRIN (PRF) IN THE TREATMENT OF BILATERAL INTRABONY DEFECTS

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ABSTRACT

Introduction: Periodontitis is an inflammatory disease of the supporting tissues of teeth caused by specific group of microorganisms resulting in destruction of periodontal ligament, alveolar bone with pocket formation, recession or both.

Aim: To evaluate clinical and radiographic outcome of Nanocrystalline hydroxyapatite (NcHA) bone graft in combination with Advanced platelet rich fibrin(A-PRF) and Platelet rich fibrin (PRF) in the treatment of bilateral intrabony periodontal defects.

Materials and Methods: Twenty intrabony defects in 9 systemically healthy patients aged between 35-55 years, were randomly assigned into Group A and Group B. The Plaque index (PI), Gingival index (GI), Probing pocket depth (PPD), Clinical attachment level (CAL), and Radiographic bone fill were recorded. In Group A NcHA bone graft with A- PRF was placed and in the Group B NcHA and PRF was placed, Post operative recall was done at 7th day, 1st month, 3rd month, and 6th month.

Results: Statistically significance with p values of < 0.0001was observed for all clinical parameters at group A, Group B pre operatively and post operatively with more significance observed in Group A (NcHA and A-PRF).

Conclusion: Group A which was treated with NcHA and A-PRF showed better results when compared with NcHA and PRF in Group B.

INTRODUCTION

Periodontitis is an inflammatory disease of the supporting tissues of teeth caused by specific group of microorganisms resulting in destruction of periodontal ligament, alveolar bone with pocket formation, recession or both.The clinical feature that distinguishes periodontitis from gingivitis is the presence of clinically detectable attachment loss which is often accompanied by periodontal pocket formation and changes in density and height of alveolar bone [1].

Hence regeneration is often the objective of therapy in patients with severe bone loss. The desired goals for reconstruction of the lost periodontal structures are using bone grafts and GTR membranes. Recent advances in bone grafting that have contributed to increased predictability include the following:

i.) Improved procurement and availability of bone graft material. ii.) Improved methods to treat diseased root structures iii.) Better understanding of the cell biology of wound healing iv.)Application of principles of GTR and use of growth factors to enhance wound healing [2] in recent years there has been an increased demand for nano materials in treatment of periodontal defects. Different alloplastic bone grafts are being developed with nanoscale particles. The most popular ones to date are Nanohydroxyapatite bone grafts, which has been introduced for augmentation procedures in intrabony defects due to its biocompatibility, bioactivity, osteoconductivity, non-toxicity and non-inflammatory nature[3].

Platelet-rich fibrin (PRF) a second generation PRP obtained from blood without adding anticoagulants by using a standard protocol 2700 rpm, 12 minutes[4]. Contains several different

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growth factors, platelets, B- and T-lymphocytes, monocytes, stem cells, neutrophilic granulocytes and other cytokines .

Advanced-PRF is a fourth generation PRF, obtained by slightly modifying the time and spin rate protocol i.e. 1500 rpm, 14 minutes centrifugation time. Consists of unique source of hematopoietic stem cells (HSCs), T and B-lymphocytes. A-PRF has a greater concentration and more homogenous distribution of monocytes which are believed to play a key role in bone formation. And also with distribution of platelets more evenly throughout the entire clot[4].

Aim

To compare and evaluate the efficacy of Nanocrystalline hydroxyapatite bone graft with Advanced platelet rich fibrin and Platelet rich fibrin in the treatment of bilateral intrabony periodontal defects.

MATERIALS AND METHODS

The present study was a split mouth study, selected sites were randomly divided into two groups by coin toss method. In Group A mucoperiosteal flap elevation followed by placement of Nanocrystalline hydroxyapatite graft with A-PRF and in Group B mucoperiosteal flap elevation with Nanocrystalline hydroxyapatite graft and PRF was done in patients undergoing periodontal therapy at the department of periodontology from St Joseph dental college Eluru, for a duration of 6 months with a sample size of 9 systemically healthy patients aged between 35 to 55 years with moderate to advanced chronic periodontitis, bilateral clinical and radiographic evidence of angular defects were included and Radiographic examination was done using standardized paralleling technique with the digital radiovisiography (RVG), the size of the defect was calculated using the RVG software X ray Vision®Apetryx.

- Patients with presence of two almost identical interproximal intrabony defects of ≥4mm one on either side of the arch based on radiographic observations.
- Patients with moderate to severe periodontitis, diagnosed on the basis of bleeding on probing, probing depth, and clinical attachment loss.

Exclusion Criteria

- Patients with history of known systemic diseases.
- People who take anti inflammatory drugs, antibiotics or within the previous 3 months.
- Pregnant and lactating mothers.
- Smokers.
- People having insufficient platelet count for PRF preparation.

Before the study an informed consent was taken and ethical committee clearance was obtained from St. Joseph dental college, Eluru. A general assessment of selected subjects was made through, clinical examination, history and routine investigations. All subjects were treated with the initial phase I therapy involving oral hygiene instructions, scaling and root planning and Cinical parameters like • Plaque index (Sillness and Loe) 5. Gingival Index (Loe and Sillness) 6. Probing depth (PD) and clinical attachment level (measured using an occlusal stent which was fabricated for positioning of the periodontal probe, Williams probe, Hufriedy) was recorded from baseline, 1, 3 and 6 month. Just prior to surgery, intravenous blood from antecubital vein was collected in 10-ml sterile tube without anticoagulant and immediately centrifuged in centrifugation machine at 2700 rpm, 12 minutes for PRF and for A- PRF at 1500 rpm for 14 minutes for platelet rich fibrin preparation, later a small section of both the PRFs was sent for histological examination which was done using H&E staining method.

Table 1 Comparision of mean values and standard deviation of plaque index, gingival index, probing depth ,clinical attachment level, radiographic measurement at baseline and after 6 months in group a and group b.

S.No	Clinical Parameters	Group A		P value	Group B		P value
		Baseline	6 months		Baseline	6 months	
1	**PI**	2.73±0.34	1.72±0.363	0.0001(s)	2.78±0.23	1.66±0.5	0.0004(s)
2	**GI**	2.7±0.43	1.4±0.52	0.0001(s)	2.7±0.43	1.88±0.41	0.0012(s)
3	**PPD**	6.6±1	4±0.707	0.0001(s)	6.77±0.97	5.22±0.97	0.0001(s)
4	**CAL**	9.22±0.97	6±1	0.0001(s)	8.66±0.86	7.22±0.97	0.0012(s)
5	**RM**	8.55±1.23	4.77±0.66	0.0001(s)	9±1.32	7±1	0.0001(s)

***S: Significant**

Inter group comparision of mean values and standard deviation of plaque index, gingival index, probing depth ,clinical attachment level, radiographic measurement at baseline and after 6 months in group a and group b.

S.No	Clinical Parameters	PRE OP		P value	POST OP		P value
		GROUP A	GROUP B		GROUP A	GROUP B	
1	**PI**	2.73±0.34	2.78±0.23	0.6923(NS)	1.72+0.363	1.66±0.5	0.7909(NS)
2	**GI**	2.7±0.43	2.7±0.43	0.9999(NS)	1.4+0.52	1.88±0.41	0.0391(S)
3	**PPD**	6.6±1	6.77±0.97	0.8141(NS)	4+0.707	5.22±0.97	0.0076(S)
4	**CAL**	9.22±0.97	8.66±0.86	0.2187(NS)	6+1	7.22±0.97	0.0182(S)
5	**RM**	8.55±1.23	9±1.32	0.4721(NS)	4.77+0.66	7±1	0.0001(S)

*** NS:Non Significant**
S:Significant

Inclusion Criteria

- Patients having pocket depth of > 6 mm.

Histological examination

After centrifugation, the clots (A-PRF, PRF) were carefully removed and the red blood cell fraction was cut carefully such

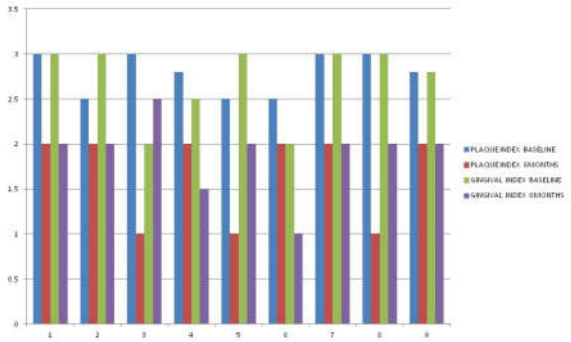
that the bottom of the fibrin clot was not disturbed and damaged this. Fibrin-based clot with the buffy coat and part of the RBC was subsequently fixed with 10% paraformaldehyde solution for 24 hours, the samples were chemically processed in an alcohol series and xylene. For further microscopic analysis paraffin embedding was performed, and 6 sections of 2 to 4 pm thickness, are affixed on charged glass slides. The samples underwent deparaffinization and rehydratation process before staining by immersing in xylene followed by ethanol, later the samples were histologically stained with standard protocols for hematoxylin and eosin (H&E) and Masson-Goldner's trachoma technique 4

Surgical Procedure

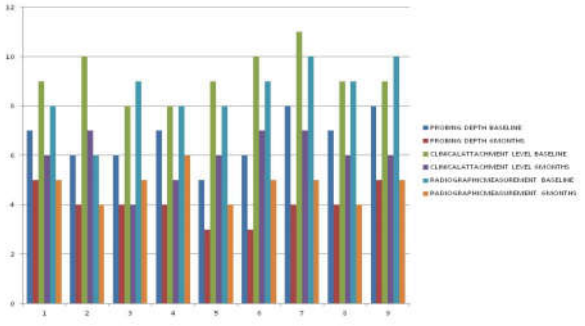
Local anesthesia was injected at the site of surgery, intra crevicular incision was given followed by mucoperiosteal flap elevation, the area was degranulated, and irrigation was done with saline solution, defect was isolated. Nanocrystalline hydroxyapatite graft was placed in small increments in the defect and condensed until the defect was filled. A- PRF and PRF was placed respectively in group A and in group B, direct loop suturing was done. Following this a non eugenol periodontal pack was applied and post operative instructions were given.

Statastical Analysis

All the results were tabulated and analyzed using Graph pad prism 6.0. Comparison within and between the studied groups were done with Paired‘ t’ and unpaired ‘ t’ test respectively.



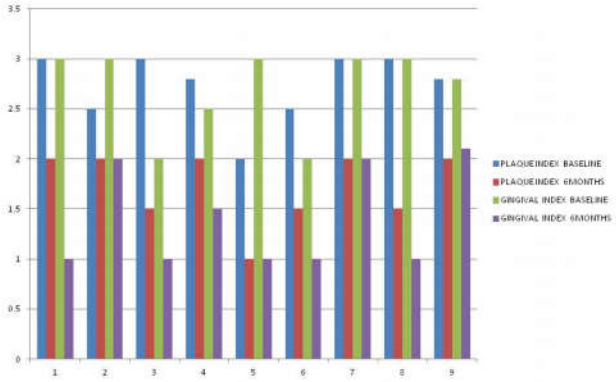
Graph 1 Site A Plaque And Gingival Index At Baseline And 6 Months



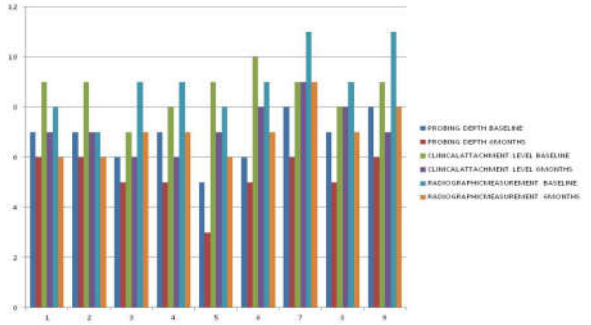
Graph 2 Site a pocket probing depth, clinical attachment level, radiographic measurement at baseline and 6 month.

RESULTS

The results in the present study achieved a reduction in the mean Plaque and Gingival index Probing depth ,Clinical attachment level and radiographic measurement scores at the treated sites at baseline and after 6 months which was statiscally significant with P values <0.0001 for paired‘ t’ test. In table 1.The Mean and SD values of Group A at baseline and after 6 months for PI was(2.73+0.34 ,1.72+0.363), GI (2.7+0.43, 1.4+0.52) , PD (6.6+1, 4+0.707), CAL (9.22+0.97, 6+1), RM (8.55+1.23, 4.77+0.66) and in Group B , PI (2.78+0.23 ,1.66+0.5), GI (2.7+0.43, 1.88+0.41), PD (6.77+0.97, 5.22+0.97), CAL(8.66+0.86 ,7.22+0.97), RM (9+1.32 ,7+1). In table 2.Unpaired‘ t’ test with p values of > 0.0001 for all clinical parameters at group A, group B pre operatively which was non significant and p value of < 0.0001 for GI, PD,CAL,RM at group A and B post operatively which was statiscally significant. The values at Group A and B pre operatively for PI was (2.73±0.34, 2.78±0.23), GI (2.7±0.43, 2.7±0.43) PD (6.6±1, 6.77±0.97) CAL (9.22±0.97, 8.66±0.86) RM (8.55±1.23, 9±1.32) and post operatively PI values was (1.72+0.363, 1.66±0.5) , GI (1.4+0.52, 1.88±0.41) PD (4+0.707, 5.22±0.97), CAL (6+1, 7.22±0.97) RM (4.77+0.66, 7±1).



Graph 3 Site B Plaque And Gingival Index At Baseline And 6 Months



Graph 4 Site b pocket probing depth, clinical attachment level, radiographic measurement at baseline and 6 month.

DISCUSSION

Chronic periodontitis in individual patients with severe attachment loss can be treated successfully with a variety of methods that are often combined. These patients are identified by clinical and radiographic evidence of severe destruction of the periodontium. Common clinical findings include severe horizontal or vertical bone loss, probing depths or attachment loss of >7mm,advanced tooth mobility, class 1 or class 2 furcation involvement and pathological migration of teeth. Hence regeneration is the main objective of therapy in these patients with severe bone loss. Periodontal bone grafts have been used for many years to treat chronic periodontitis with severe attachment loss. The objectives of periodontal bone graft

are a) Probing depth reduction b) Clinical attachment gain c) Regeneration of new bone, cementum, and periodontal ligament[2].

Comparision of protocols and advantages of platelet concentrates

Platelet concentrates	Protocol	Advantages
Prf(plasma rich fibrin)	12 min, 2700 rpm	Fibrin meshwork with entrapped platelets-
A-prf(advanced plasma rich fibrin)	14 min, 1500 rpm	More amount of granulocytes/ monocytes, 50% more bmp
L-prf(leucocyte rich plasma fibrin)	10 min ,3000 rpm	15 times >vegf and 2 > tgf beta
I-prf(injectable plasma rich fibrin)	3 min, 7oo rpm	Injectable and in liquid form coagulates immediately after injection .results still in infancy

Bone substitutes in the form of Nano hydroxyapatite can be used successfully to fill osseous defect as it has a dvantages like close contact with surrounding tissues, quick resorption characteristics and large number of molecules on the surface [7.] Platelet concentrates PRF and A-PRF was used in our study of which

Platelet-rich fibrin (PRF) which is obtained from human blood with a protocol of 2700 rpm for 12 minutes in conjunction with bone grafts offers several advantages like enhancing wound sealing, bone growth and haemostatis.

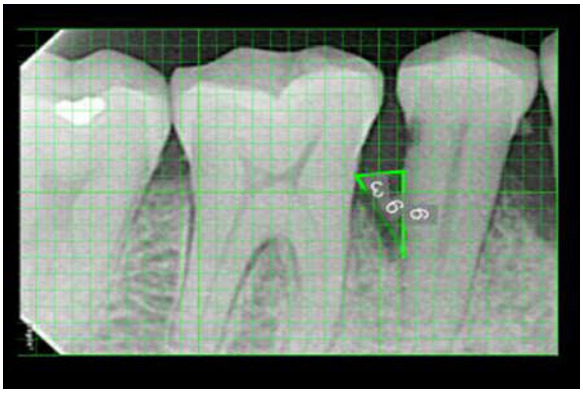


Fig 1 (a)

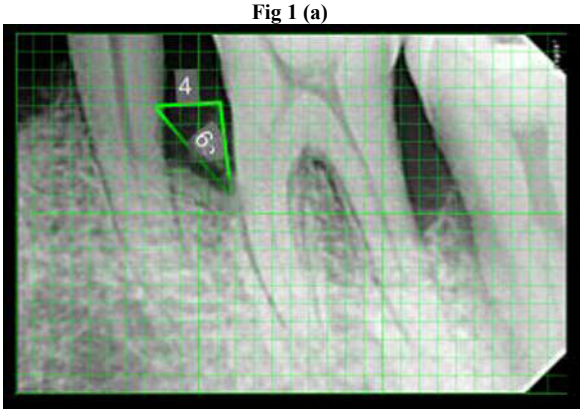


Fig 1 (b)

Fig 1 (a): pre operative intrabony defect in radiograph (group a with ncha and a-prf).
(b): pre operative intrabony defect in radiograph (group b with ncha and prf).

It consists of fibrin mesh at the end of the spin that includes growth factors . Bone stimulation by PRF is that it acts as a provisional extra cellular matrix with fibrin, fibronectin and thrombospondin, which induces an early and fast vascularization[8.]

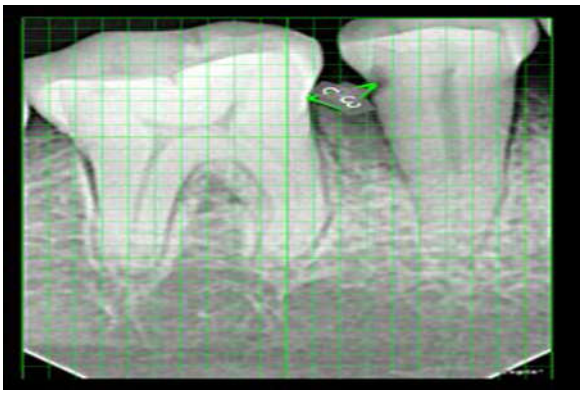


Fig 2 (a)

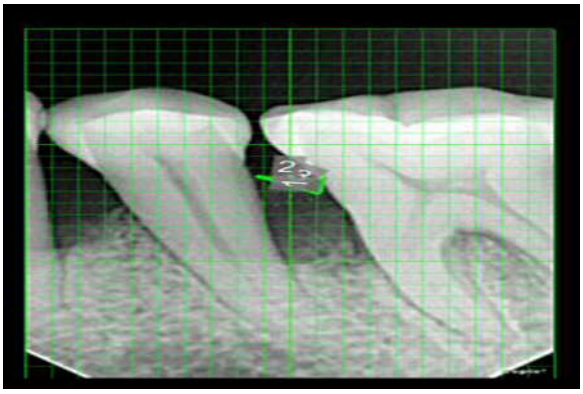


Fig 2 (b)

Fig 2 (a), (b): post operative intrabony defect in radiograph (group a and group b).

Advanced platelet rich fibrin (A-PRF) a fourth generation PRF which is obtained with a standard protocol of 1500 rpm for 14 minutes, consists of more amount of monocytes, platelets and BMPS, which was the major advantage of A-PRF but with an increase in centrifugation time i.e 14 minutes when compared with PRF[4].

The future is now more exciting with new protocol i-PRF (700 rpm for 3 min)which is in liquid and injectable form and coagulates immediately after the injection with same PRF concept without adding anticoagulants, but the results of this I-PRF is still in infancy[8].

The present study was designed to compare and evaluate the clinical and radiographic outcomes obtained following treatment of intrabony periodontal defects with Nanocrystalline hydroxyapatite graft with A -PRF on one side and with Nanocrystalline hydroxyapatite and PRF on other side.

Both treatment modalities treated with NcHA with A-PRF and PRF achieved a reduction in the mean Plaque and Gingival index scores from baseline to 6 months which was statistically significant. And the results was in accordance with the study conducted by Enas ahmed *et al* [3], was opposite with the study conducted by Yukna *et al*. and Meffert *et al*[7] who reported statistically insignificant results for plaque and

gingival index, and reported no effect on the plaque index when Hydroxy apatite(HA) graft was used in treatment of intrabony defects, but this reduction in PI and GI in both groups in the present study may be attributed to mechanical oral hygiene procedures which was maintained throughout the study.

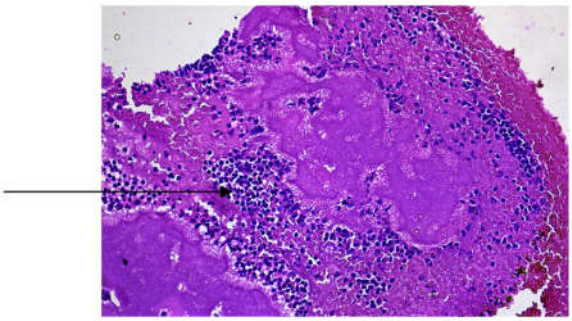


Fig 3 A (Close Network of Cells In A-PRF)

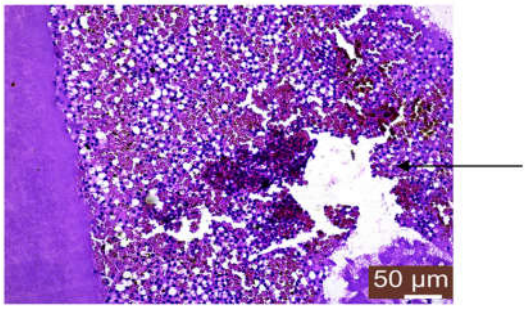


Fig 3 B (Wide distributiton of Cells In PRF)

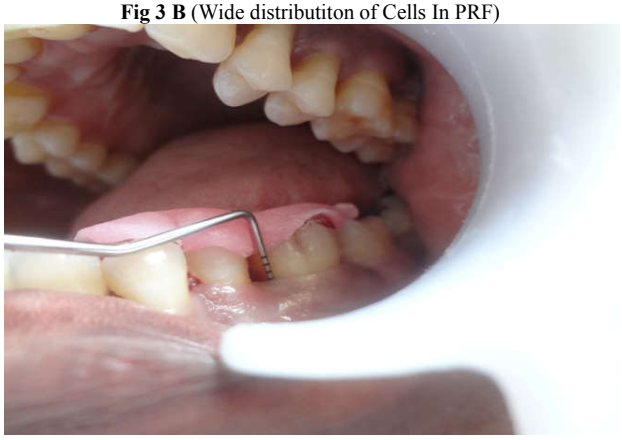


Fig 4 (A)

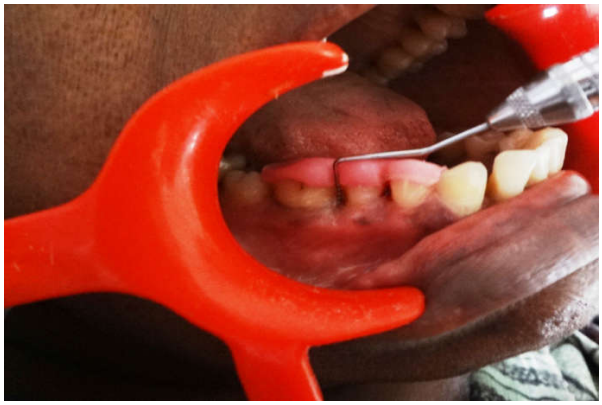


Fig 4 (B)

Fig 4 (A), (B) Pre Operative Probing Depth (Group A And Group B).

A significant improvement in PPD reduction, CAL gain, and radiographic defect fill was observed in both Group A which is treated with A-PRF and NcHA and in Group B with PRF and NcHA from Baseline to 6 months postoperatively, this results was positive in accordance with the study conducted by Enas ahmed *et al* [3] who evaluated the clinical efficacy of NcHA graft with PRF, with the study conducted by Kasaj *et al*[3] who evaluated the clinical efficacy of NcHA paste in intrabony defects, vijendra *et al* [9] who evaluated the efficacy of NcHA graft combined with collagen membrane in treatment of intrabony defects, Kiliac *et al*[3] demonstrated that the combination of HA collagen bone graft with ePTFE membrane has shown reduction in PDD and CAL,.

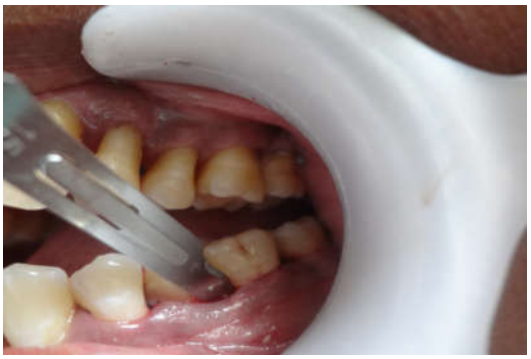


Fig 5 (A)

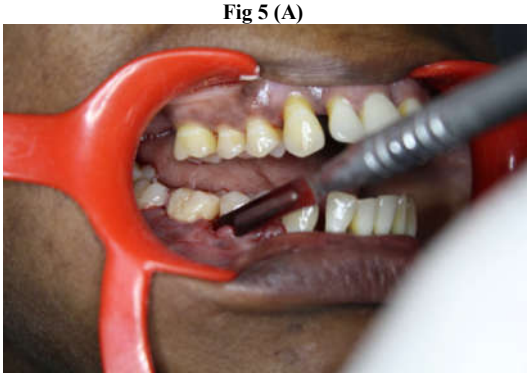


Fig (5) B

Fig 5 (A), (B) Intracrevicular Incisions (Group A And Group B).

In accordance with the radiological observations of Okuda and Scabbia *et al* 7 who evaluated the depth of the intrabony defect radiographically after a period of 12 months reported significant gain in the bone height and reduction of the depth of the defect. Eickholz *et al* [7] also reported a decrease in the size of the defect from baseline to 3 and 6 months, also the difference from 3 months to 6 months was statistically significant, Wenzel *et al* [9] reported that there was no increased bone fill between 6 and 12 months which may support the six month radiographic analysis of the present study.

But group A (A- PRF with NcHA graft) had shown more significant results than group B (PRF and NcHA graft). This difference might be due to the fact that A- PRF is a rich source of hematopoietic stem cells (HSCs), monocytes, T and B - lymphocytes, neutrophilic granulocytes, and platelets[4], seen equally distributed through out the clot when compared to PRF that had enhanced bone and soft tissue regeneration in group A. However further long term post operative observations may be needed for evaluation and stability on the clinical outcome of the results.

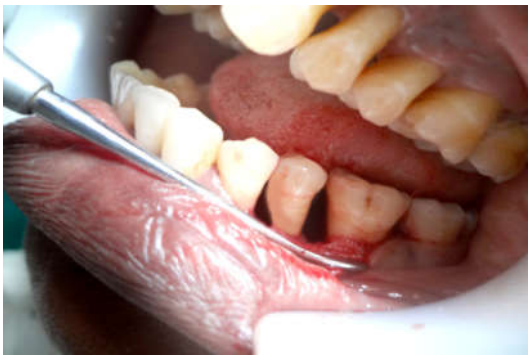


Fig 6(A)

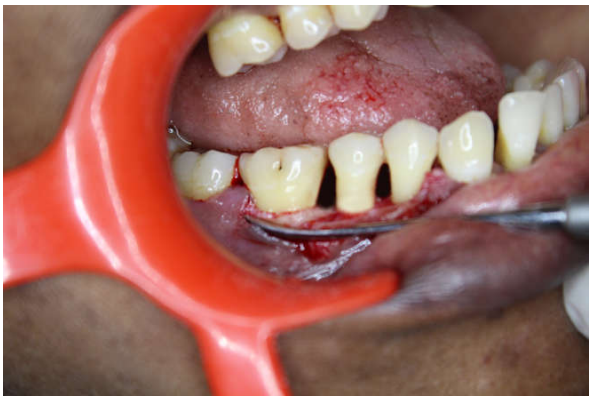


Fig 6(B)

Fig 6 (A), (B) mucoperiosteal flap elevation (group a and group b).

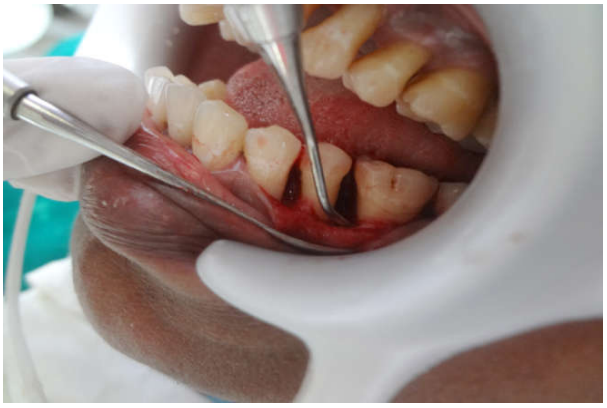


Fig 7(A)

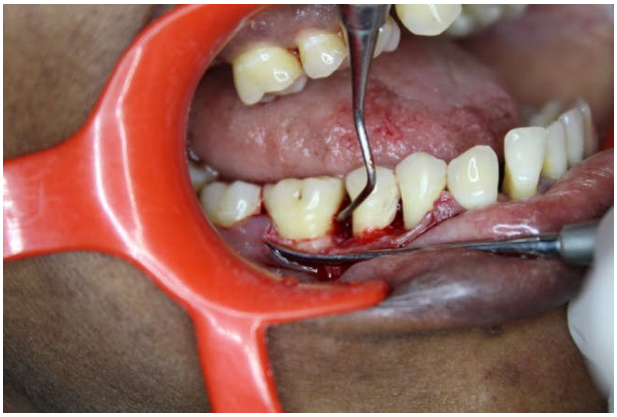


Fig 7(B)

Fig 7 (a), (b) degranulation (group a and group b).

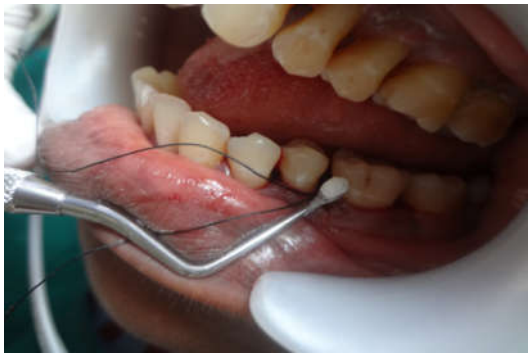


Fig 8 (A)

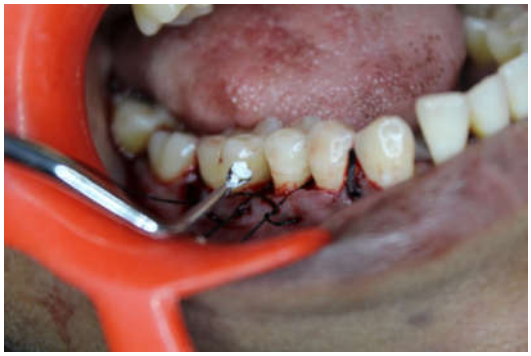


Fig 8(B)

Fig 8 (A), (B) Placement of Nanohydroxyapatite Graft Into Defect Site (Group A And Group B).

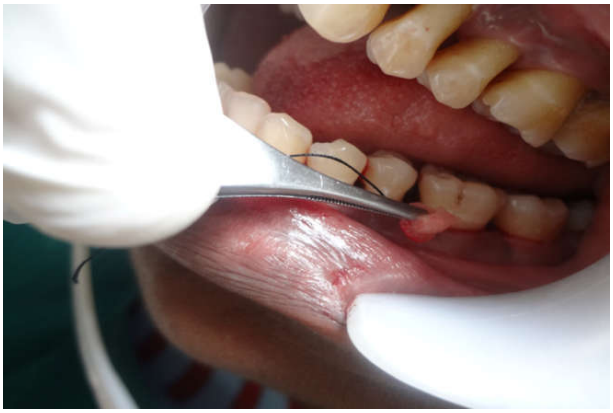


FIG 9(A)

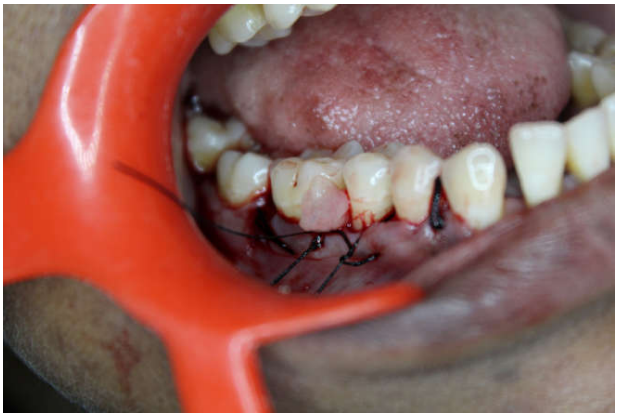


FIG 9 (B)

FIG 9 (A) Placement of A-Prf Into The Defect Site (Group A).
(B) Placement of Prf Into The Defect Site (Group B).

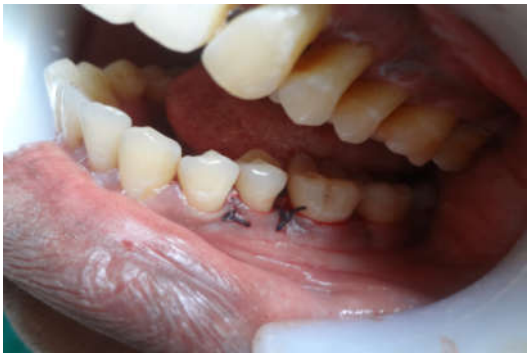


Fig 10(A)

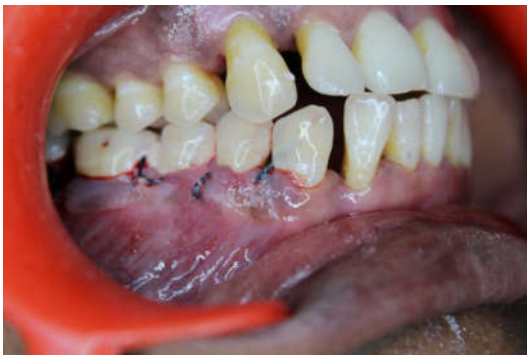


Fig 10 (B)

Fig 10 (A), (B) Suturing Of The Defect Site (Group A and Group B).

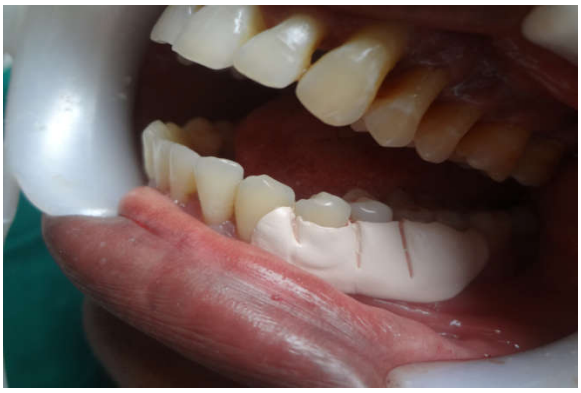


Fig 11(A)

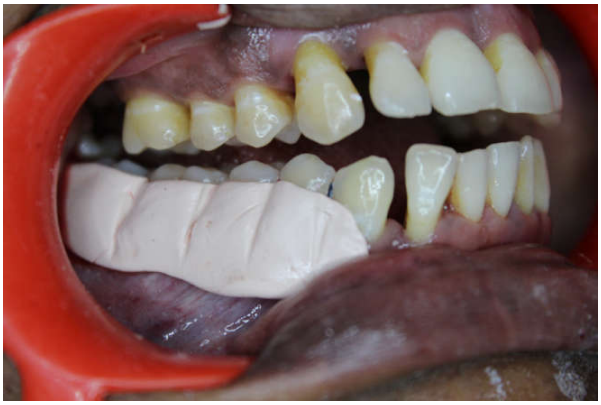


Fig 11(B)

FIG 11 (A), (B) Periodontal Dressing (Group A And Group B).

Fig 12(A)

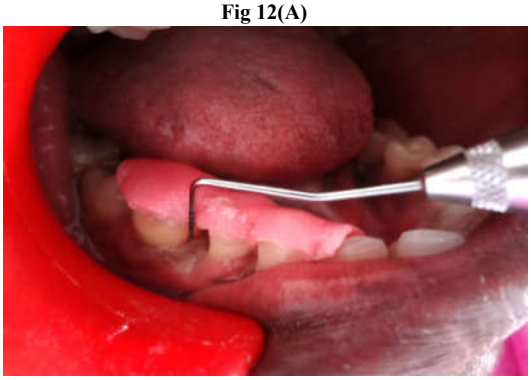


Fig 12(B)

Fig 12 (A), (B) Post Operative Probing Depth (Group A And Group B).

Limitations

It is a study of its kind that has been conducted with a shorter duration of time and sample size with no histological analysis done which is the most reliable method for assessing periodontal regeneration.

Future Aspects

With the results obtained from the above study A-PRF when combined with NcHA had shown better regenerative properties, better bone gain, bone fill, and graft stabilization. However this combination therapy can be used with larger clinical trials, sample size and Longer duration for further evaluating the regenerative properties of Nano hydroxyapatite and A-PRF.

CONCLUSION

Nanocrystalline hydroxyapatite can be used as promising bone graft in the treatment of intrabony defects. Adjunctive use of PRF membrane (A- PRF and PRF) in combination with Nanocrystalline hydroxyapatite bone graft showed statistically significant reduction in all clinical parameters and radiographic measurements but the group treated with Advanced PRF and Nanocrystalline hydroxyapatite graft showed more reduction in probing depth, clinical attachment level and radiographic measurements compared to group treated with PRF and Nanocrystalline hydroxyapatite graft.

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