Original Article

Extracellular matrix containing nanocomposite bone graft in periodontal regeneration – A randomized controlled clinical and radiographic evaluation

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Abstract:

Background: The study aims to evaluate the effect of adding extracellular matrix (ECM) component – natural collagen to nanocrystalline hydroxyapatite (nHA) bone graft in the treatment of intrabony defect in chronic periodontitis patients. **Materials and Methods:** Forty chronic periodontitis patients having at least one intrabony defect were treated surgically by open flap debridement and the defect grafted (Group A: 20 sites grafted with nHA with natural collagen and Group B: 20 sites grafted with nHA). Plaque index, gingival index, probing pocket depth (PPD), clinical attachment level (CAL), and radiographic defect depth (RDD) were evaluated. **Results:** The mean PPD reduced from 7.6 ± 0.88 at baseline to 4.45 ± 0.69 and 2.60 ± 0.6 at 3 and 6 months, respectively, in Group A. In Group B, the mean PPD reduced from 7.5 ± 0.89 at baseline to 4.95 ± 0.60 and 2.65 ± 0.59 at 3 and 6 months, respectively. The mean CAL reduced from 7.75 ± 0.85 at baseline to 5.05 ± 0.76 and 3.6 ± 0.68 at 3 and 6 months, respectively, in Group A. In Group B, the mean CAL reduced from 7.70 ± 0.86 at baseline to 5.8 ± 0.7 and 3.75 ± 0.64 at 3 and 6 months, respectively. The mean RDD reduced from 8.13 ± 0.78 and 8.12 ± 0.83 at baseline to 4.27 ± 0.66 and 3.94 ± 0.5 after 6 months in Groups A and B, respectively. After 3 months, a statistically significant reduction in mean PPD and CAL values was noted in Group A while the results were comparable after 6 months. **Conclusion:** The effectiveness of nHA composite during initial healing phase (3 months) can be attributed to the presence of ECM-collagen in bone graft matrix.

Key words:

Bone grafts, collagen, extracellular matrix, nanocrystalline hydroxyapatite, periodontal regeneration

INTRODUCTION

Bone is a stratified nanocomposite tissue comprising of hydroxyapatite crystals within a collagenous matrix.[1] Intrabony defects resulting from chronic periodontitis are treated surgically using various bone grafts with the aim of achieving periodontal regeneration.

The key to regeneration is to stimulate the coordinated cascades of events that result in integrated tissue formation. Biomimetic scaffolds having structural architecture similar to extracellular matrix (ECM) of bone are promising as bone graft materials. ECM scaffolds create favorable microenvironment that supports cell infiltration, survival, and differentiation.[2] They also act as suitable templates for tissue regeneration.[3]

Collagen, the most abundant body protein, is the most commonly used ECM-derived material for fabricating biomimetic scaffold.[4] The triple helix structure with intrafibrillar, interspersed apatite crystals creates a microenvironment to promote tissue regeneration.[5]

Nanocrystalline hydroxyapatite (nHA) has an extremely high number of molecules on the surface of the material which helps in accelerated substitution by vital bone.[6] Sybograf, Eucare

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Pharmaceuticals Private Limited, Chennai, India, is a novel nHA bioceramic material, synthetically prepared using biomimetic process technology [Figure 1a].

When combined with collagen, it forms a nanocomposite scaffold that mimics native bone tissue. These collagen-based nanocomposites function as an interface of natural and synthetic molecules, to facilitate regeneration and improved functional and biologic properties.[7] Sybograf-C, Eucare Pharmaceuticals Private Limited, Chennai, India, is synthetic nHA with collagen of fish origin [Figure 1b].

No study till date has been conducted to compare and evaluate periodontal regenerative efficacy by addition of an ECM component – collagen to nHA bone graft. The present study attempts to evaluate clinically and radiographically, the effects of nHA when used alone and in combination with natural collagen (nanocrystalline hydroxyapatite composite [nHAC]) in the treatment of human periodontal intrabony defects in patients with chronic periodontitis.

MATERIALS AND METHODS

The present study was a randomized controlled clinical trial conducted during 2015–2018. A total of forty patients for the proposed study who were diagnosed with chronic periodontitis classified on the basis of the 1999 consensus classification of periodontal disease[8] were selected from the outpatient department. The study was approved by the institutional ethical committee and was conducted in accordance with the Helsinki Declaration of 1975, as revised in 2013 and registered in Clinical Trials Registry-India (CTRI) (CTRI/2018/01/011302).

A thorough history followed by clinical examination was conducted. Patients who were systemically healthy, nonsmokers, and nonpregnant women who exhibited deep periodontal pockets (>5 mm) were selected, and intraoral periapical (IOPA) radiographs were examined. Those patients under medications known to cause gingival enlargement, on anticoagulant therapy, or immunocompromised were excluded. Those who showed radiographic evidence of vital teeth and vertical/angular bone loss (3 or 2 wall defects) in affected sites were selected to be enrolled in the study, subject to satisfactory oral hygiene maintenance during Phase I (full-mouth supra- and subgingival scaling and root planing) of treatment. On assessment after 6 weeks post-Phase I, patients were enrolled after recording an informed video consent. If the patient desired to discontinue the treatment procedure during the study, he/she was allowed to do so.

Plaque index (PI)[9] and gingival index (GI)[10] were the clinical parameters recorded at baseline (after Phase I), 1, 3, and 6 months postsurgery. Probing pocket depth (PPD) and clinical attachment level (CAL) were measured using UNC-15 Probe University of North Carolina 15, Hu-Friedy®, Chicago, IL, USA, and acrylic stent with grooves to standardize the probe angulation and position at baseline (after Phase I), 3, and 6 months postsurgery.

The intrabony defects in the maxilla or mandible from the selected patients were randomly divided by computerized automated randomization method into Group A and Group B.

IOPA radiographs of the selected sites were taken using long cone paralleling technique at baseline, immediate postsurgery, and 6 months postsurgery. Radiographic defect depth (RDD) reduction (D6) was evaluated by comparing the radiographic depth of the defect. This was determined using the cementoenamel junction and the most apical extension of the intrabony destruction where the periodontal ligament space still retained its normal width before treatment, as the fixed reference points at baseline (A0 and B0) and after 6 months (A6 and B6) for Groups A and B, respectively. The measurements on the radiograph were made using the measurement scale in digital software (Adobe Photoshop, USA).

D6 = (A0−A6) and (B0−B6).[11]

Perioral scrub with 10% povidone-iodine was followed by 10 ml of 0.2% chlorhexidine prerinse. Surgery was performed under local anesthesia by infiltrations and/or nerve blocks using injection 2% lignocaine hydrochloride with 1:100,000 epinephrine. After adequate anesthesia, a crevicular incision was made using Bard-Parker blade handle and Swanson and Morton No. 15 surgical blade. A full-thickness mucoperiosteal flap was reflected using the periosteal elevator and defect thoroughly debrided using Gracey curettes, Hu-Friedy®, Chicago, IL, USA. Root planing, site irrigation, and evaluation were carried out.

Before the placement of bone graft, 4-0 black-braided silk nonabsorbable suture material was passed through the buccal and lingual interdental papillae using 3/8 circle, 20G, reverse cutting needle, and the sutures were left untied.

For Group A patients, nHAC was mixed with saline in a sterile dappen dish and placed in the intrabony defect [Figure 2]. The same was done using nHA for Group B patients [Figure 3]. The surgical site was later closed using interrupted loop sutures.

An immediate postsurgical IOPA radiograph was taken. The area was protected with a noneugenol dressing (Coe-Pak™, GC, USA) for 1 week.

All patients were prescribed systemic antibiotic tablet doxycycline hydrochloride 200 mg once for the first day followed by 100 mg once daily for 6 days. A combination of anti-inflammatory analgesic tablet containing ibuprofen (400 mg) and paracetamol (325 mg) was prescribed thrice daily for 3 days. 10 ml of 0.2% chlorhexidine rinse twice daily for 7 days was given, and patients were instructed for postoperative oral hygiene maintenance.

After 1 week following surgery, the periodontal dressing and sutures were removed. Recall appointments were made after 1, 3, and 6 months postsurgery. Clinical and radiographic parameters were assessed at each recall visit [Figure 4].

The recorded parameters were statistically analyzed using parametric tests (paired *t*-test to compare intragroup *P* values and unpaired *t*-test to compare intergroup *P* values). To compare the effect of different time intervals on PPD and CAL, the repeated measure one-way analysis of variance test followed by Tukey's multiple comparison test was conducted.

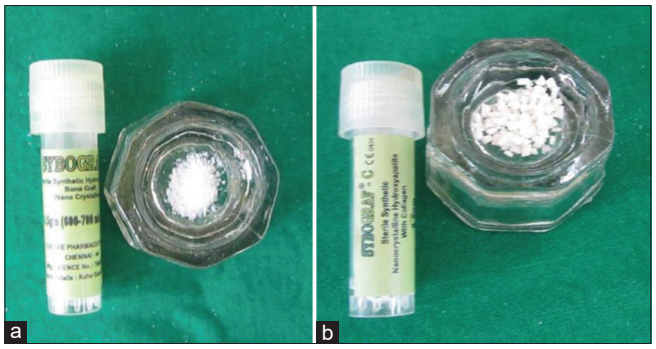

Figure 1: (a) Nanocrystalline hydroxyapatite bone graft – Sybograf, Eucare Pharmaceuticals Private Limited, Chennai, India; (b) Nanocrystalline hydroxyapatite with natural collagen bone graft – Sybograf C, Eucare Pharmaceuticals Private Limited, Chennai, India

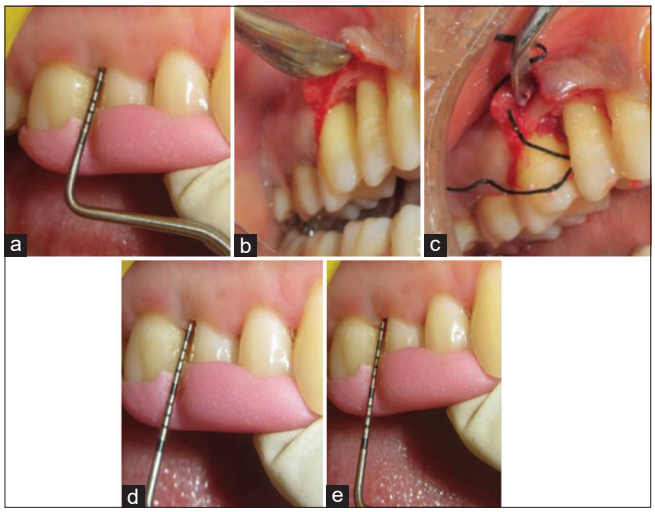

Figure 3: (a) Group B: Open flap debridement surgery – Presurgical vertical probing pocket depth; (b) Debridement of intraoral defect; (c) Placement of Bone Graft – Sybograf, Eucare Pharmaceuticals Private Limited, Chennai, India, in intrabony defect after presuturing; (d) Vertical probing depth after 3 months; (e) Vertical probing depth after 6 months

RESULTS

The study consisted of 40 patients (24 males and 16 females) having mean age of 33.5 years (21–56 years) with 27 in the maxilla (67.5%) and 13 in the mandible (32.5%) – 25 on the mesial side (62.5%) and 15 on the distal side (37.5%). Twenty-three were on the right side (57.5%) and 17 were on the left side (42.5%). The power of the study was 0.8 (that is 80%) at 95% confidence interval.

PI and GI showed reduction from baseline values when compared after 1, 3, and 6 months. When results of both the groups were compared, no statistically significant difference was observed ($P < 0.05$) [Tables 1 and 2].

The effect of treatment on PPD was seen as reduction in mean PPD values in both the groups. The mean PPD reduction after 6 months in Group A was 5.0 ± 0.28 mm and in Group B was 4.85 ± 0.30 mm [Table 3]. Pair-wise comparison within groups showed that the difference was significant among baseline-3 months and baseline-6 months in both the groups [Tables 4 and 5].

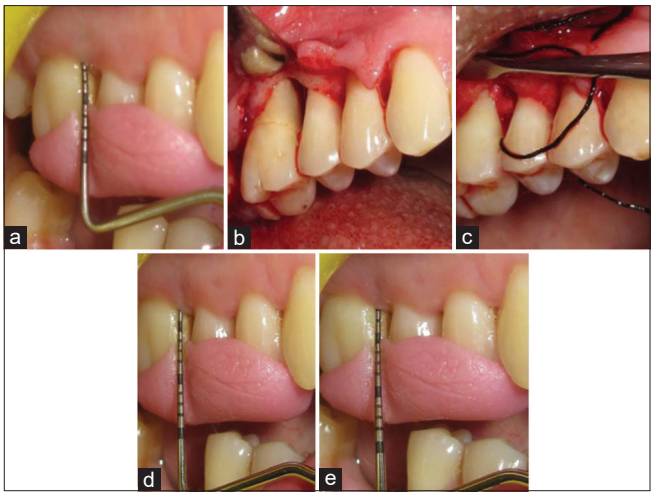

Figure 2: (a) Group A: Open flap debridement surgery – Presurgical vertical probing pocket depth; (b) Debridement of intraoral defect. (c) Placement of bone graft – Sybograf C, Eucare Pharmaceuticals Private Limited, Chennai, India, in intrabony defect; (d) Vertical probing depth after 3 months; (e) Vertical probing depth after 6 months

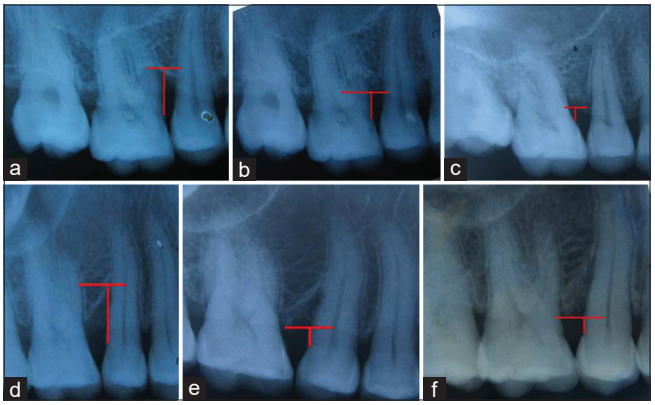

Figure 4: (a) Group A: Presurgical/baseline intraoral periapical radiograph; (b) Group A: Immediate postoperative intraoral periapical radiograph; (c) Group A: 6 months postsurgery intraoral periapical radiograph; (d) Group B: Presurgical/baseline intraoral periapical radiograph; (e) Group B: Immediate postoperative intraoral periapical radiograph; (f) Group B: 6 months postsurgery intraoral periapical radiograph

Table 1: Comparison of the effect on plaque index in Group A and Group B

Time interval	Base line	1month	3 months	6 months
P	0.3064	0.9571	0.2160	0.0637
Significantly different? ($P<0.05$)	No	No	No	No

P – Probability Coefficient

Table 2: Comparison of the effect on gingival index in Group A and Group B

Time Interval	Base line	1month	3 months	6 month
P	0.8294	0.8304	0.8218	0.9358
Significantly different? ($P<0.05$)	No	No	No	No

P – Probability Coefficient

Table 3: Effect on intervention on probing pocket depth in Group A and Group B

Time Interval	Group A			Group B		
	Baseline	3 months	6 months	Baseline	3 months	6 months
Minimum	6.00	3.00	2.00	6.00	4.00	2.00
Maximum	9.00	5.00	4.00	9.00	6.00	4.00
Mean	7.60	4.45	2.60	7.50	4.95	2.65
Standard Deviation	0.88	0.69	0.60	0.89	0.60	0.59
Reduction of PPD (Baseline - 6 months)		5.0±0.28			4.85±0.30	

PPD – Probing Pocket Depth

Table 4: Tukey's multiple comparison test in Group A: Significant reduction in probing pocket depths over time

Test details	Mean difference	q	Significant?
Baseline vs 3 months	3.15	22.4	Yes
Baseline vs 6 months	5	35.55	Yes
3 months vs 6 months	1.85	13.15	Yes

q – Universal term used in Tukey's statistical test

Table 5: Tukey's multiple comparison test in Group B: Significant reduction in probing pocket depths over time

Test details	Mean difference	q	Significant?
Baseline vs 3 months	2.55	20.38	Yes
Baseline vs 6 months	4.85	38.76	Yes
3 months vs 6 months	2.3	18.38	Yes

q – Universal term used in Tukey's statistical test

Table 6: Comparative analysis of probing pocket depth in Group A and Group B

Time Interval	Base line	3 months	6 month
P	0.1625	0.0141	0.7715
Significantly different? (*P*<0.05)	No	Yes	No

Unpaired *t*-test statistically significant reduction in Group A as compared to Group B at 3 months *P*<0.05, *P* – Probability Coefficient. It is a measure of the probability that an observed difference could have occurred just by random chance

When compared with each other, Group A had statistically significant lower values (4.45 ± 0.69 mm) of PPD than Group B (4.95 ± 0.60 mm) only after 3 months ($P = 0.0141$) ($P < 0.05$) [Table 6].

Similarly, the effect of treatment was seen as reduction in mean CAL values in both the groups. The mean gain in CAL after 6 months in Group A was 4.15 ± 0.17 mm and in Group B was 3.95 ± 0.22 mm [Table 7]. Pair-wise comparison showed that the difference was significant among baseline-3 months and baseline-6 months in both the groups [Tables 8 and 9]. When compared with each other, Group A had statistically significant lower values (5.05 ± 0.76 mm) of CAL than Group B (5.80 ± 0.70 mm) only after 3 months ($P = 0.0024$) ($P < 0.05$) [Table 10].

The mean RDD in Group A was 8.13 ± 0.78 mm at baseline and 4.27 ± 0.66 mm at 6 months from baseline and in Group B was 8.12 ± 0.83 mm at baseline and 3.94 ± 0.50 mm at 6 months from baseline [Table 11]. When comparison was made between the two groups, the decrease in RDD was statistically not significant when observed at baseline and after 6 months ($P < 0.05$) [Table 12]. The mean D6 in Group A was 3.86 ± 0.78 mm and in Group B was 4.18 ± 0.69 mm which when compared were not statistically significant ($P < 0.05$) [Table 13].

DISCUSSION

Periodontal regenerative therapy not only arrests the inflammatory process but also reconstructs the lost periodontium.[12] To direct this process in desired direction, it is necessary to control ECM accumulation and cell behavior during healing.[13] ECM components play a critical role in morphogenesis.[2] Natural polymers, for example, collagen, offer great bioactivity and biocompatibility because of similarity with native ECM.[14]

Bone is a discontinuously reinforced composite. The reinforcing phase is nanoparticles of a calcium phosphate mineral, and the matrix in which the nanoparticles are embedded consists primarily of Type 1 collagen. Type I collagen represents the lowest hierarchical level of bone tissue.[15] Collagen-based scaffolds (nHAC) prepared by biomimetic process simulate the ECM of native bone.[16] The collagen in the graft used in this study is of fish origin. Marine collagen possesses biocompatibility, biodegradability, easy extractability, water solubility, safety, low immunogenicity, and low production costs.[17]

Due to the limitations of the availability and morbidity of autologous grafts,[18] synthetic bone grafts are desirable. Calcium phosphate ceramics (e.g., hydroxyapatite) have proven to be biocompatible and are well tolerated by soft tissue without eliciting an inflammatory or foreign body response.[19] Hydroxyapatite biomaterials are complex calcium phosphates which resemble bone mineral in their chemical composition [$Ca_{10}(PO_4)_6(OH)_2$] and have a calcium-to-phosphate ratio of 1.67.[20]

The use of nanosized hydroxyapatite results in a significant increase in protein absorption and osteoblast adhesion on the nHA particles, thereby providing stimulus to bone healing by increasing the binding of nHA bone graft to the bone.[21,22] In addition, nHA is a strong promoter of angiogenesis.[23] nHA is a stimulator of periodontal ligament cells, thereby contributing to periodontal tissue regeneration.[24]

The present study demonstrated no clinical evidences of undesirable local and systemic responses and showed positive clinical and radiographic changes after using both nHAC and nHA.

As the baseline data for PI, GI, PPD, and CAL assessed were similar, it can be stated that the final differences between the groups were not influenced by initial defect characteristics. Thus, the posttreatment results can be compared.

Table 7: Effect on intervention on clinical attachment level in Group A and Group B

Time Interval	Group A			Group B		
	Baseline	3 months	6 months	Baseline	3 months	6 months
Minimum	6.00	3.00	2.00	6.00	5.00	3.00
Maximum	9.00	6.00	5.00	9.00	8.00	5.00
Mean	7.75	5.05	3.60	7.70	5.80	3.75
Standard Deviation	0.85	0.76	0.68	0.86	0.70	0.64
Reduction of CAL (Baseline – 6 months)		4.15±0.17			3.95±0.22	

CAL – Clinical Attachment Level

Table 8: Tukey's multiple comparison test in Group A: Significant reduction in clinical attachment level values over time

Test details	Mean difference	q	Significant?
Baseline vs 3 months	2.7	16.56	Yes
Baseline vs 6 months	4.15	32.29	Yes
3 months vs 6 months	1.45	8.344	Yes

q – Universal term used in Tukey's statisical test

Table 9: Tukey's multiple comparison test in Group B: Significant reduction in clinical attachment level values over time

Test details	Mean difference	q	Significant?
Baseline vs 3 months	1.9	12.42	Yes
Baseline vs 6 months	3.95	26.45	Yes
3 months vs 6 months	2.05	18.89	Yes

q – Universal term used in Tukey's statisical test

Table 10: Comparative analysis of clinical attachment level in Group A and Group B

Time Interval	Base line	3 months	6 month
P	0.8547	0.0024	0.4767
Significantly different? (*P*<0.05)	No	Yes	No

Unpaired *t*-test statistically significant reduction in Group A as compared to Group B at 3 months *P*<0.05

Plaque control exhibited by patients directly affects the long-term success,[25] whereas the gingival health status is depicted by GI. A general trend of progressive decline in PI and GI scores over the duration of the study was seen and is in agreement with a study conducted by Krejci *et al*.[26] This could be due to the repeated reinforcement of oral hygiene habits in recall visits and overall general improvement in periodontal parameters. Since histology was not practically feasible, clinical outcomes such as reduction of PPD, gain in CAL, and radiographs are used to assess the clinical efficacy of regenerative bone grafts.

PPD indicates volumetric subgingival area potentially harboring putative periodontal pathogens. Plaque removal efficacy decreases as pocket depth increases.[27] Thus, PPD correlates directly with long-term maintenance of periodontal health. In the present study, reduction in PPD posttreatment can be attributed to the reduction in inflammation and shrinkage of pocket wall. It can also occur due to combination of gain in clinical attachment and posttreatment gingival recession.

The positive correlation between the gain in CAL and the gain in vertical bone height has led to the use of CAL as an important outcome variable to evaluate regeneration.[28] Gain in CAL was statistically significant in Group A during the early postoperative period of 3 months but was comparable with Group B after 6 months. This finding is in correlation with significant reduction of PPD observed after 3 months in Group A. The reason for the early improved clinical parameters (PPD and CAL) in Group A can be attributed to collagen content in the graft, which provides the necessary tissue ECM at a site of healing. Early healing corresponds to reduced incidence of complications during regeneration. This early clinical resolution of intrabony defects is in coherence with findings by Panday *et al*. and Thanikasalam *et al*.[29,30]

The recapitulation of formative process is facilitated by stimulation of coordinated tissue healing response.[30] The nanocomposite graft (nHAC) is composed of the two main components of bone – collagen and hydroxyapatite, and exhibits a hierarchical structure that resembles the one found in natural bone.[1] Collagen served not just as a template but also initiated and propagated mineralization independent of the matrix vesicles.[31] Collagen scaffold materials facilitate the growth of vasculature into the material and provide an ideal environment for bone formation.[32] The nanostructure of the mineralized collagen scaffolds was demonstrated to influence their biological activities, such as initial cell adhesion, morphology, proliferation, and further osteogenic differentiation and mineralization.[16] Marine-derived collagen peptides demonstrated bioactivity for alveolar bone regeneration *in vitro* by upregulating osteogenic markers and promoting viability of primary human periodontal ligament cells.[33]

The advantage of addition of collagen in the graft leading to early integration of this graft with cancellous bone has been demonstrated in an animal model.[34] The bone-forming ability of the graft was demonstrated to be enhanced after addition of collagen to hydroxyapatite.[35] The superiority of bone formation after addition of collagen in nHA has been demonstrated radiographically in extraction sockets.[29]

Although the RDD reduction was statistically nonsignificant when compared between the two groups, the values in Group B showed greater reduction as compared to Group A. This nonsignificant intergroup finding can be attributed to the presence of similar composition of the graft material (nHA). Furthermore, slight greater RDD reduction observed in Group B can be due to the particulate consistency of nHA (Group B) having better radiographic visualization compared to the sponge-like consistency of nHAC (Group A). This finding was in accordance with *in vivo* animal study by Liu *et al*. which, though demonstrated lesser radio-opacity, histologically displayed partially degraded composite graft and new bone ingrowth for bioinspired collagen apatite nanocomposites.[36]

Table 11: Radiographic defect depth changes at baseline and after 6 months and radiographic bone fill in Group A and Group B after 6 months

Time Interval	Group A			Group B		
	Baseline	6 months	Radiographic bone fill	Baseline	6 months	Radiographic bone fill
Minimum	6.82	3.21	2.34	6.21	2.89	2.43
Maximum	9.65	5.23	4.95	9.48	4.84	5.25
Mean	8.13	4.27	3.86	8.12	3.94	4.18
Standard Deviation	0.78	0.66	0.78	0.83	0.50	0.69

Table 12: Comparison of radiographic defect depth changes in Group A versus Group B after 6 months

Time Interval	Baseline	6 months
P	0.8123	0.1733
Significantly different ? (*P*<0.05)	No	No

Unpaired *t*-test no statistically significant difference between Group A and Group B. *P* – Probability Coefficient

Table 13: Comparison of radiographic bone fill (D6) between Group A and Group B after 6 months

P	0.1734	Difference of Radiographic bone fill between Group A and Group B is NOT statistically significant
Statistically Significant? (*P*<0.05)	no	

Unpaired t-test no statistically significant difference in the amount of bone fill P<0.05. *P* – Probability Coefficient

This study demonstrated overall PPD reduction with postsurgery PPD of ≤4 mm and mean CAL gain of ≥3 mm in both the groups. This outcome is regarded as clinically relevant periodontal regeneration in accordance with combined outcome measure criteria proposed by Trombelli *et al*.[37]

Larger sample size, longer follow-up duration, paired/split-mouth design, and cone-beam computed tomography radiographic test would be desirable.

CONCLUSION

Both graft materials studied displayed satisfactory clinical and radiographic outcomes and were biocompatible. It can be concluded that the addition of collagen in nHA bone graft does enhance the early clinical parameters, but the results were comparable in both the groups after 6 months. Long-term evaluation of the sites is recommended to further track the healing and evaluate the sustainability of the results.

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Conflicts of interest

There are no conflicts of interest.

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