Clinicoradiographic evaluation of hyaluronan-nano hydroxyapatite composite graft in the management of periodontal infrabony defects

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Abstract:
Background: Hyaluronan is a naturally occurring polysaccharide in the extracellular matrix of the connective tissue. It imparts antibacterial and osteogenic properties to the nano hydroxyapatite bone graft (NHA). Aim: This study aims to evaluate the efficacy of hyaluronan-NHA (H-NHA) composite in the treatment of infrabony defects in chronic periodontitis patients. Materials and Methods: Eight systemically healthy chronic periodontitis patients with 32 graftable sites were included in the study. After flap reflection and debridement, the defect sites in the test quadrant were filled with H-NHA graft and those in the control quadrant were filled with NHA graft alone. Clinical parameters such as probing pocket depth (PPD) and clinical attachment level (CAL) were assessed at baseline and 3, 6, 9, and 12 months; bone probing depth (BPD) and radiographic parameters such as amount and percentage of defect depth reduction (DDR, PDDR) and change in alveolar crest level (ALR) were assessed at baseline and 6 and 12 months. Statistical Analysis Used: Descriptive statistics, one-way analysis of variance, and Student’s t-test were used for statistical analysis. Results: At the end of 12 months, H-NHA group showed a greater reduction in PPD (5.06 ± 0.582 mm), BPD (4.22 ± 0.371 mm), and gain in CAL (4.00 ± 0.421 mm) as compared to the NHA group (3.21 ± 0.648 mm, 3.21 ± 0.047 mm, and 2.86 ± 0.127 mm, respectively). In addition, DDR, PDDR, and ALR were better in H-NHA group (1.92 ± 0.647 mm 48.22 ± 31.561 mm, and 1.22 ± 0.808 mm, respectively) as compared to the NHA group (1.14 ± 0.602 mm, 20.14 ± 25.349 mm, and 0.89 ± 0.626 mm, respectively). Statistically significant improvements in all the parameters were seen in the test sites when compared to control sites at 12 months. Conclusion: H-NHA composite graft can be considered a promising periodontal regenerative material.

Key words:
Bone regeneration, chronic periodontitis, clinical parameters, hyaluronan-nano hydroxyapatite composite graft, infrabony defects, nano hydroxyapatite bone graft

INTRODUCTION

Periodontal disease presents a major health problem globally and is one of the two most important oral diseases contributing to the global burden of chronic diseases.[1] It is the major cause of tooth loss which adversely affects the quality of life of an individual.[2]

Over the years, various treatment modalities such as the use of local drug delivery agents, barrier membranes, bone replacement graft materials, biologically active regenerative materials, or a combination of any of the above methods have been successfully used for regeneration of new periodontal attachment.[3]

The chemical and structural similarity of synthetic hydroxyapatite (HA) [Ca10(PO4)6(OH)2] to natural bone mineral, biocompatibility, tolerance, and biologically active property makes it an ideal bone substitute.[4] Although osteophilic and osteoconductive, HA is not osteogenic or osteoinductive. It only acts as a scaffold for bone formation.[5] Furthermore, it lacks antibacterial action, which can impair the bone healing process leading to failure of surgical operation.[6] Another disadvantage of using HA bone graft is the scattering of graft particles.[7]

One way to overcome these disadvantages is by adding a second material to the graft, which will provide it with additive properties, making

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it a composite graft.\[8-10\] Hence, in this study hyaluronan was added to nano HA (NHA) bone graft.

Hyaluronan (hyaluronic Acid) is a viscous, natural linear polysaccharide that presents in the extracellular matrix of connective tissue, synovial fluid, and other tissues.\[11\] Medium- and low-molecular weight hyaluronan has a bacteriostatic effect against Aggregatibacter actinomycetemcomitans, Prevotella oris, and Staphylococcus aureus.\[12\] It enhances proteoglycan production, reduces the production and activity of pro-inflammatory mediators and matrix metalloproteinases, and has antioxidant properties.\[13\] It has bone induction characteristics similar to osteogenic substances bone morphogenetic protein-2 and osteopontin.\[14\] High-molecular weight hyaluronan accelerates bone formation by chemotaxis, proliferation, and successive differentiation of mesenchymal cells.\[14\]

Thus, mixing hyaluronan to NHA can impart osteogenic and antibacterial properties to the bone graft. It gives a putty-like consistency to the graft particles, thus enhancing its handling and working characteristics.

Considering the beneficial effects of hyaluronan, this study aims to assess the efficacy of hyaluronan-NHA composite graft (H-NHA) in comparison with NHA graft alone, in the treatment of infrabony defects in chronic periodontitis patients.

**MATERIALS AND METHODS**

Based on a study by Okuda et al.,\[15\] group standard deviations of 1.700 and 1.200 and a significance level (alpha) of 0.05000 were taken. Using PASS software (Power Analysis and Sample Size software program, licensed by NCSS, LLC, Kaysville, Utah, USA), a sample size of 18 sites per group was determined. This gave a power of 81% to detect a difference of 1.400 in clinical attachment level (CAL) changes at 12 months between the null and alternate hypothesis using a two-sided t-test.

Ten systemically healthy patients (six females and four males) with bilateral defects were chosen for this 12-month study from the outpatient section of our institution. During flap surgeries, a total of 38 graftable sites were found, among which 20 were taken as test and 18 as control. Two patients were lost to follow-up. Thus, at the end of 12 months, a total of 8 patients with 32 graftable sites (18 test and 14 control) were analyzed [Figure 1].

The study protocol was approved by the Institutional Ethical Committee and Review Board. The study is registered under Clinical Trials Registry-India (Reg No: CTRI/2017/11/010591).

Prior written informed consent was obtained from all the participants of the study.

Inclusion criteria consisted of systemically healthy male and female patients with chronic generalized periodontitis, 30–55 years of age, and having contralateral infrabony pockets measuring ≥6 mm; radiographic evidence of bilateral vertical/ angular defects; and consenting patients who were ready for regular follow-up. Patients with a history of periodontal or antibiotic therapy in the past 6 months, pregnant/lactating women, smokers, medically compromised, or under therapeutic regimen that may alter the probability of soft tissue and bone healing were excluded from the study.

Following initial examination, all participants received oral hygiene instructions. Full-mouth scaling and root planing was performed under local anesthesia. On re-evaluation, full-mouth indices were recorded and only patients showing optimum oral hygiene status were scheduled for surgery.

The selected quadrants in each individual were divided randomly by a computer-generated method into test and control, according to split-mouth design. All graftable sites in test and control quadrants were considered as test and control sites, respectively. Test sites were treated using H-NHA composite graft and control sites with NHA graft alone. All clinical and radiographic assessments were done by an experienced periodontist who was blinded to the type of bone graft used during the surgery.

Full-mouth plaque index (PI), gingival index (GI), gingival bleeding index (BI), probing pocket depth (PPD), and CALs were measured at 3, 6, 9, and 12 months. Site-specific PPD and CAL were checked at 3, 6, 9, and 12 months and bone probing depth (BPD) was checked at baseline and 6 and 12 months.

PPD, CAL, and BPD were measured using graduated periodontal probe (UNC PCP-15 periodontal probe, Hu-Freidy, Chicago, IL, USA) and standardized customized acrylic stents to provide reproducible insertion axis. Local anesthesia was administered before the measurement of BPD. The acrylic stent was positioned and the probe was inserted along the vertical locating grooves to the crest of the alveolar bone. The apical margin of the stent was considered as the fixed reference point (FRP) for clinical measurements. Only one site representing the same deepest point of the defect was included.

Following are the abbreviations used in the formulas:

PPD: Probing pocket depth, BOP: Base of pocket, FRP: fixed reference point, GM: Gingival Margin, CAL: clinical Attachment level, BPD: Bone Probing Depth, CEJ: Cementoenamel Junction

The following measurements were recorded:

\[
PPD = (FRP \text{ to BOP}) - (FRP \text{ to GM})
\]

\[
CAL = (FRP \text{ to BOP}) - (FRP \text{ to CEJ})
\]

\[
BPD = (FRP \text{ to BPD}) - (FRP \text{ to GM})
\]

Intraoral periapical radiographs with super imposed X-ray mesh gauge were taken using paralleling technique. The X-ray mesh gauge had fine grids measuring 1 mm × 1 mm. The radiographs were taken at baseline and 6 months and 12 months, by one radiologist with standardized parameters, exposure, and processing.

The cementoenamel junction (CEJ) was taken as the FRP on the radiograph FRP\(_{r} \). The landmarks, CEJ (FRP\(_{r} \)), alveolar crest (AC), and base of defect (BOD) were marked on the
radiographic image obtained and the distance from FRP to BOD and AC was measured.

\[ \text{Defect depth (Dd) = (FRP to BOD) − (FRP to AC)} \]

Changes in AC level (ALR) = (FRP to AC at baseline) − (FRP to AC at recall time interval)

\[ \text{Defect depth reduction (DDR) = \text{Initial defect depth} − \text{Defect depth at recalled time interval}} \]

\[ \text{Percentage of DDR (PDDR) = (DDR/baseline defect depth) × 100} \]

In a similar study by Ballini et al.,[16] 0.5cc of autologous bone graft was mixed with two parts of hyaluronan fibers to get a packable consistency of graft material. Keeping this as the basis, during each surgery, the required amount of synthetic NHA bone graft (Sybograf™, Eucare, Chennai, Tamil Nadu, India) was dispensed in a dappen dish and hyaluronan gel (Gengigel® Prof Syringes, Ricerfarma, Milan, Italy) was added in an incremental manner. A packable consistency of composite graft was obtained by mixing equal parts of bone graft and hyaluronan gel (for example – 0.5 g of bone graft with 0.5 ml of hyaluronan gel).

After adequate local anesthesia, sulcular incisions were given on the facial and lingual aspects and full-thickness mucoperiosteal flaps were reflected. The defect was cleared of granulation tissue and thoroughly root planed using area-specific curettes (Gracey currets, Hu-Friedy). The defect sites in the test quadrant were filled with H-NHA graft [Figure 2] and those in the control quadrant were filled with NHA graft alone [Figure 3]. Flaps were approximated using a sling and interrupted (4-0) Black Braided Silk Sutures.

A noneugenol periodontal dressing (Coe-Pak, GC America, Chicago, IL, USA) was placed at the surgical site.

Antibiotics (amoxicillin 500 mg, thrice daily for 5 days) and analgesics (paracetamol and aceclofenac combination twice daily for 3 days) along with 0.2% chlorhexidine digluconate rinses (twice daily for 7 days) were prescribed to all the patients. Suture removal was done 7 days after the surgery. The patients were recalled at 3, 6, 9, and 12 months.

Statistical analysis was done using SPSS version 10.0.5 (SPSS Inc., IBM, Armonk, NY, USA) package. The results were averaged (mean ± standard deviation) for continuous data and number and percentage for dichotomous data were determined. Normality assumption of the data was tested.
using the Shapiro–Wilk test. In this study, data were normally distributed and parametric test was used to compare between the groups. The values obtained from the clinical and radiographic data were subjected to statistical analysis with one-way analysis of variance and Student’s t-test. \( P < 0.05 \) was considered statistically significant.

RESULTS

At the end of 12 months, 8 patients with 32 graftable sites (18 test and 14 control sites) were analyzed. Of the 8 patients, 6 were female and 2 were male, with a mean age group of \( 36.9 \pm 7.492 \) [Table 1].

All patients showed uneventful postoperative healing with good soft-tissue response to both the treatments.

There was a significant reduction in full-mouth scores of PI (from \( 2.53 \pm 0.301 \) to \( 0.61 \pm 0.164 \)), GI (from \( 2.34 \pm 0.311 \) to \( 0.40 \pm 0.131 \)), BI (from \( 74.07 \pm 10.536 \) to \( 24.29 \pm 4.758 \)), PPD (from \( 7.24 \pm 0.885 \) to \( 2.63 \pm 1.061 \)), and gain in CAL (from \( 5.88 \pm 1.008 \) to \( 1.91 \pm 0.645 \)) from baseline to 12 months [Table 2].

Intragroup comparison of site-specific PPD, CAL, and BPD showed a statistically significant improvement over a period of 12 months for both the groups (\( P < 0.001 \)) [Table 3].

PPD and CAL did not show any statistically significant difference between the test and control groups at baseline. At 12 months, PPD and CAL were \( 2.00 \pm 0.767 \) and \( 1.33 \pm 1.188 \), respectively, in the test group and \( 3.50 \pm 1.787 \) and \( 2.71 \pm 1.326 \), respectively, in the control group. The test sites showed a statistically significant improvement as compared to the control sites at 12 months (\( P = 0.003 \) and \( P = 0.004 \), respectively) [Table 3].

Baseline BPD was \( 6.61 \pm 1.614 \) in the test group and \( 6.71 \pm 1.139 \) in the control group. At 12 months, it was \( 2.39 \pm 1.243 \) in the test group and \( 3.50 \pm 1.092 \) in the control group. The test sites showed a statistically significant improvement as compared to the control sites at 12 months (\( P = 0.013 \)) [Table 3].

The radiographic parameters- DDR, PDDR and ALR, were considered to be 0.0 at baseline. At 12 months, DDR and PDDR were \( 1.92 \pm 0.647 \) and \( 48.22\% \pm 31.561\% \), respectively, in the test group and \( 1.14 \pm 0.602 \) and \( 20.14\% \pm 25.349\% \), respectively, in the control group. The test sites showed a statistically significant improvement at 12 months as compared to control sites (\( P = 0.002 \) and \( P = 0.008 \), respectively). ALR did not show a statistically significant change in both the groups at any time interval [Table 3].
Table 1: Descriptive statistics of demographic data

| Parameter | n  | Age (years) | Number of site | H-NHA (test site) | NHA (control site) | Maxillary site | Mandibular site |
|-----------|----|-------------|----------------|-------------------|--------------------|---------------|---------------|
|           |    | 30-47       | 32             | 18                | 14                 | 16            | 16            |
| Mean±SD   |    | 36.9±7.492  | 2.13±1.246     | 2.25±1.488        | 2.00±1.000         | 3.20±1.643    | 2.29±0.951    |
| Minimum   |    | 24          | 1              | 1                 | 1                  | 2             | 1             |
| Maximum   |    | 47          | 5              | 5                 | 5                  | 6             | 4             |

n - Number participants; SD - Standard deviation; H-NHA - Hyaluronan-nano hydroxyapatite composite graft; NHA - Nano hydroxyapatite graft; SD - Standard deviation

Table 2: Full-mouth plaque index, gingival index, bleeding index, probing pocket depth, and clinical attachment level

| Parameter | Time interval | Mean±SD | P  |
|-----------|---------------|---------|----|
| PI        | Baseline      | 2.53±0.301 | <0.001* |
|           | 3 months      | 1.40±0.428 |    |
|           | 6 months      | 0.85±0.307 |    |
|           | 9 months      | 0.80±0.151 |    |
|           | 12 months     | 0.61±0.164 |    |
| GI        | Baseline      | 2.34±0.311 | <0.001* |
|           | 3 months      | 0.96±0.267 |    |
|           | 6 months      | 0.63±0.219 |    |
|           | 9 months      | 0.49±0.113 |    |
|           | 12 months     | 0.40±0.131 |    |
| BI        | Baseline      | 74.07±10.336 | <0.001* |
|           | 3 months      | 59.65±9.789 |    |
|           | 6 months      | 41.04±7.287 |    |
|           | 9 months      | 35.51±8.564 |    |
|           | 12 months     | 24.29±4.758 |    |
| PPD       | Baseline      | 7.24±0.885  | <0.001* |
|           | 3 months      | 6.18±1.295  |    |
|           | 6 months      | 4.60±1.364  |    |
|           | 9 months      | 3.78±1.631  |    |
|           | 12 months     | 2.63±1.061  |    |
| CAL       | Baseline      | 5.88±1.008  | <0.001* |
|           | 3 months      | 4.62±1.024  |    |
|           | 6 months      | 3.64±1.306  |    |
|           | 9 months      | 2.78±1.279  |    |
|           | 12 months     | 1.91±0.645  |    |

*Statistically significant at P<0.05. PI - Plaque index; GI - Gingival index; BI - Bleeding index; PPD - Probing pocket depth; CAL - Clinical attachment level; SD - Standard deviation; P - P value

The defects were divided according to their types into two-walled, three-walled, and combined defects. All three types of defects were present in both the groups [Table 4].

In two- and three-walled defects, PDDR showed no statistically significant difference between the two groups at 6 and 12 months.

In combined defects (three and two walled and two and one walled), PDDR at the test site was 34.3 ± 10.066 at 6 months and 66.7 ± 38.188 at 12 months. At control sites, the values were 5.0 ± 8.367 at 6 months and 13.8 ± 22.094 at 12 months. The test group showed statistically higher bone fill than the control group.

**DISCUSSION**

In this study 0.8% high-molecular weight hyaluronan gel was added to commercially available NHA graft to study its enhanced regenerative properties in the treatment of infrabony defects in chronic periodontitis patients, over a period of 12 months using a split-mouth design. The split-mouth design was adopted in this study to avoid interpatient variability and provide similar healing conditions for both the groups.[17] Machtei et al. in 1996 found a significant reduction in probing depth and gain in attachment 1 year after treating furcation defects using barrier membranes. These results were maintained for over 4 years. Thus, they stated that endpoint measurement for regenerative studies should be done at least 12 months postoperatively.[10] Thus, a duration of 12 months was chosen for this study.

To the best of our knowledge, no clinical study has been published using hyaluronan-NHA composite graft in chronic periodontitis patients. Studies using hyaluronan with autologous bone graft showed better results at 12 months.[19] and with demineralized bone graft for sinus augmentation are available in the literature.[19]

The primary outcome variables in our study include clinical parameters such as PPD, CAL, and BPD and radiographic parameters such as DDR and PDDR. The secondary outcome variables include full-mouth PI, GI, and BI.

Reduction in full-mouth scores of PI, GI, PPD, and gain in CAL suggested overall improvement of gingival and periodontal health over a period of 12 months.

The results of site-specific PPD and CAL showed that test sites had statistically better improvement, which was in accordance with a study by Ballini et al.[19] where a combination of hyaluronic acid and autologous bone graft showed a gain in clinical attachment over a period of 24 months.

Adrian Kasaj et al.[20] in 2008, compared the efficacy of NHA paste to open flap debridement over a period of 6 months where NHA paste significantly improved clinical outcomes. Nanostructured materials have an extremely high number of molecules on the surface of the material. This provides greater surface area for binding of NHA to bone and leads to bone healing by stimulation of osteoblastic activity.

The present study showed a significant mean reduction in BPD in both the groups over a period of 12 months. However, on the intergroup comparison, the test sites showed significantly better results at 12 months.

Kim et al.[21] stated that BPD measurement is a reliable method to estimate the regenerated bone level following periodontal treatment as there is a minimal difference between BPD and actual bone level. Hence, BPD has been evaluated in this study.

Considering the patient’s acceptability, discomfort, and ethical reasons, surgical re-entry was not considered for evaluation of bone formation. Clinical and radiographic parameters were thus assessed.
A linear change in radiographic fill was detected using consecutive radiographs. The long-cone paralleling technique was used. Conventional intraoral periapical radiographs with an X-ray mesh gauge were used. The X-ray mesh with grid markings of 1 mm was used to make linear measurements easy.

The DDR and PDDR were significant at 6 and 12 months for both the groups. On intergroup comparison, the test sites showed a statistically significant improvement as compared to the control at 12 months. There was no significant change in ALR in both the sites at all intervals.

These results were in accordance with a study by Ballini A et al.,[16] which showed satisfactory bone fill radiographically, over a period of 24 months, in infrabony defect sites treated with hyaluronic acid and autologous bone graft combination.
The morphogenic effects of hyaluronic acid are due to its ability to act as a scaffold which provides a hydrated environment for the cells to undergo morphological changes. Hyaluronic acid being a vector for bone morphogenic protein stimulates bone formation.\[22\]

Varying morphology of initial defects greatly influences the treatment outcomes that are reported in various studies. In this study, some defects were two-walled defects (2.12 ± 1.690) and some were three-wall defects (1.5 ± 0.707), while others were combination defects (1.8 ± 0.837).

According to Cortellini et al.,\[23\] deep, narrow defects and two- or three-wall defects have the highest potential for regeneration when grafted. Although it is necessary to know the potential effects of this variability on the results, it is hard to control these variables in a clinical investigation. Even in the best of circumstances, it is impossible to find matched osseous defects. However, randomization may help to control this variable, which was followed in this study.

Thus, based on the clinical experience while performing the surgeries and from the statistical data, we can conclude that hyaluronan gives a cohesive mass of packable consistency when mixed with NHA bone graft and hence prevents scattering of the graft particles. Pirnazar et al.\[24\] in their study tested the bacteriostatic and bactericidal properties of hyaluronan on Streptococcus mutans, Porphyromonas, P. oris, A. actinomycetemcomitans, S. aureus, and Propionibacterium acnes. They concluded that high concentrations of the medium-molecular weight hyaluronan had the greatest bacteriostatic effect on A. actinomycetemcomitans, P. oris, S. aureus, and P. acnes strains and middle concentration of the high-molecular weight hyaluronan had the greatest overall bacteriostatic effect, inhibiting the growth of all six bacterial strains tested. Significant bacteriostatic effects were observed regardless of concentration or molecular weight of hyaluronic acid for S. aureus and to a greater extent for A. actinomycetemcomitans. Thus in our study, we can say that hyaluronan further provides an environment which promotes wound healing and bone formation due to antibacterial\[12,24\] and bone induction\[11\] properties.

However, there are a few limitations in this study. Resolution of infrabony defects with the use of bone graft was assessed by the visual increase in the radiodensity in the defect and a decrease in the defect size. These changes were quantified by calculating the number of squares on the grid. However, the nature of the regeneration cannot be inferred from the clinical and radiographic observations of the present study. Only histologic investigation can reveal the true nature of attachment, which is not possible due to ethical issues. Furthermore, only a linear method of radiographic evaluation of defect fill has been used in this study. Thus, future studies should incorporate precise radiographic evaluation showing three-dimensional bone formation, using the advanced digital imaging facilities available, with a larger sample size.

**CONCLUSION**

Within the limitation of this study, the hyaluronan-NHA composite graft showed a significant improvement in the clinical and radiographic parameters along with improved handling characteristics.

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**Conflicts of interest**

There are no conflicts of interest.

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