Original Article

Clinical and Radiographic Evaluation of Citric Acid-Based Nano Hydroxyapatite Composite Graft in the Regeneration of Intrabony Defects - A Randomized Controlled Trial

Chaurasia Priya Dayashankar, P. C. Deepika, Basavarajaiah Siddaramaiah[1]

Department of Periodontology, JSS Dental College and Hospital, Jagadguru Sri Shivarathreeshwara University, [1]Department of Polymer Science and Technology, Sri Jayachamarajendra College of Engineering, Mysore, Karnataka, India

Abstract

Background: Conventional periodontal therapy with various bone grafts has limited scope and the results are not predictable. To improve their utility, the hybridization of bioceramics and biodegradable polymers has been widely adopted to reform the mechanical properties of bone grafts. One such biodegradable polymer is POC (Poly 1,8 octanediol). Secondly, citric acid is considered as the key material in bone mineralization, which is related to the overall stability, strength and fracture resistance of bone. Hence citric acid is incorporated in a polymer and Nano hydroxyapatite to form a composite graft, for periodontal bone regeneration. This study attempts to evaluate the efficacy of citric acid based Nano-hydroxyapatite composite graft for the treatment of intrabony defects in chronic periodontitis patients over 12 months. **Methods:** A split mouth study, which consists of 10 systemically healthy patients, were randomly treated with Citric acid based Nano hydroxyapatite composite graft (test sites, n=18) or with Nano hydroxyapatite alone (control sites, n=15). Plaque index, gingival index, gingival bleeding index, probing pocket depth (PPD), clinical attachment level (CAL), bone probing depth (BPD) and hard tissue parameters such as amount of defect fill, percentage of defect fill, and changes in alveolar crest were assessed over a period of 12 months. Statistical analysis used was student's *t*-test and One-Way ANOVA. **Results:** Both test and control sites demonstrated statistically significant reduction of PD, BPD, gain in CAL and radiographic bone fill. Nevertheless the test sites showed Statistically significant improvements in all the parameters as compared to control sites at 12 months. **Conclusion:** Citric acid based Nano hydroxyapatite composite graft can be considered as a newer material for periodontal regeneration.

Keywords: *Bone regeneration, citric acid, clinical and radiographic parameters, intrabony defects, nano hydroxyapatite bone graft, polymer*

Introduction

Periodontitis is defined as "an inflammatory disease of the supporting tissues of the teeth caused by specific microorganisms or a group of specific microorganisms resulting in progressive destruction of the periodontal ligament and alveolar bone with pocket formation, recession, or both."[1] Gingivitis and periodontitis are among the most prevalent microbial diseases of humankind. The pathological hallmark of periodontitis is the destruction of the supporting structure of the teeth involved. Although clinical bleeding on probing (BOP), erythema, edema, and suppuration may be clinical signs and symptoms of periodontal disease, the diagnosis of periodontitis is made by identifying the loss of attachment with periodontal probing and intraoral radiographs.

Scaling and root planing or open flap debridement are the conventional and gold standard methods for the treatment of periodontal disease. Conventional surgical procedures have only limited potential in the regeneration of periodontal tissues, hence various types of bone grafts have been widely used to promote bone formation as well as periodontal regeneration.[2]

Autologous bone grafts are the gold standard for bone grafting procedures in a variety of fields including orthopedics, neurosurgery, and dentistry due to their superior osteogenic potential. In dentistry, it is associated with various disadvantages such as additional surgical site, another potential location for postoperative pain, and complications. It is also contraindicated in osteoporotic patients due to a significant reduction

How to cite this article: Dayashankar CP, Deepika PC, Siddaramaiah B. Clinical and radiographic evaluation of citric acid-based nano hydroxyapatite composite graft in the regeneration of intrabony defects - A randomized controlled trial. Contemp Clin Dent 2017;8:380-6.

Address for correspondence:
Dr. Chaurasia Priya Dayashankar, Department of Periodontology, JSS Dental College and Hospital, Jagadguru Sri Shivarathreeshwara University, Mysore - 570 015, Karnataka, India.
E-mail: drpriya.chaurasia@gmail.com

Access this article online

Website: www.contempclindent.org

DOI: 10.4103/ccd.ccd_213_17

Quick Response Code:

in bone quality and quantity. Thus, the development of a synthetic, readily available, and osteogenic bone substitute as an adjunct to autologous tissue graft is required and considered a great need in the clinical field.[3]

A variety of synthetic bone graft materials are used to mimic the native composition of bone. Some of these materials are hydroxyapatite (HA) and beta tricalcium phosphate (TCP). Although these materials are biomimetic and osteoconductive, their application is severely limited due to inherent brittleness and slow degradation. Thus, in the quest for searching a material to overcome these limitations, Sun *et al*.[3] and Tran *et al*. in 2014[4] developed a citric acid-based HA composite scaffold with two different polymers and checked regeneration in rat calvarias.

Citrate, a naturally occurring Krebs's cycle product, is highly conserved in native bone. Recent research has suggested that citrate plays significant roles in bone anatomy, physiology, and orthopedic biomaterial development.[4-6] The source of citrate formation is unidentified, but it is found that citrate comprises ~1.6% of the bone content, and about 80% of the total body citrate is present in the bone.[5] The bound citrate covers about 1/6th of the available apatite surface area in bone and accounts for 5.5 wt% of the organic matter in bone.[5] "Osteoblast citration" is one of the newer concepts in bone formation. It has been suggested that citrate has a role in mineralization of osteoblast in bone formation; it now becomes evident that "citration" must be included in the process of mineralization. Mineralization without "citration" will not result in the formation of normal bone, i.e., bone that exhibits its important properties such as stability, strength, and resistance to fracture. A previous research by Hu *et al*.[6] and Davies *et al*.[7] has shown that citrate molecules are strongly studded to the apatite nanocrystal surface and form bridges between mineral platelets regulating bone mineral crystallinity, which is highly related to the overall strength of bone tissue.

Thus, considering these properties of citrate in bone regeneration, the aim of the present study was to evaluate the efficacy of citric acid-based nano HA (NHA) composite graft in comparison to NHA bone graft alone in surgical management of intrabony defects, in patients with chronic periodontitis.

Materials and Methods

Study population

The participants of the study were enrolled from the outpatient department of our institution over a period of 6 months from May 2015 to November 2015. Sample size was calculated based on a study by Okuda *et al*.[8] from where mean and standard deviation was obtained to estimate sample size using 80% power and 5% level of significance. Thus, ten systemically healthy patients were chosen with bilateral defects for this 12-month study. Hence, twenty sites were selected. However, after flap reflection, we found 33 graftable sites, of which 15 were taken as control sites and 18 as test sites. The patients were selected on the following criteria: patients who were ready to sign the informed consent, systemically healthy patients, both males and females in the age range of 30–55 years having chronic generalized periodontitis, contralateral intrabony pockets measuring ≥6 mm, radiographic evidence of vertical/angular bone loss, consenting patients who were co-operative and able to come for regular follow-up, and patients with ≥20 remaining teeth.

The criteria for exclusion of the patients were as follows: pregnant/lactating women, individuals allergic to citric acid, patients who received antibiotic therapy in the previous 6 months, patients undertaken any periodontal therapy in the past 6 months, smokers, and patients medically compromised or under therapeutic regimen that may alter the probability of soft tissue and bone healing.

The sites were randomly assigned using the computer software-generated randomization method to test and control groups. The test sites were treated with citric acid-based NHA (CA-NHA) and the control sites were treated by placing NHA alone. All pre- and post-treatment clinical parameters were recorded by an examiner who was masked to the type of treatment received by the patients while another clinician provided treatment to both groups.

Clinical parameters such as full mouth plaque index (PI),[9] gingival index (GI),[10] gingival bleeding index (GBI),[11] pocket probing depth (PPD), and clinical attachment levels (CALs) were measured at baseline, 3, 6, 9, and 12 months.

Site-specific PPD and CALs were checked at baseline, 3, 6, 9, and 12 months, and bone probing depth (BPD) was checked at baseline and at 6 and 12 months. Site-specific radiographic parameters such as amount of defect fill (ADF), percentage of defect fill (PDF), and change in alveolar crestal level (ACL) were measured at baseline and at 6 and 12 months.

The primary end point of the study was to assess the bone fill, clinically as well as radiographically, over a period of 12 months.

PPD, CAL, and BPD measurements which were recorded with UNC 15 probe were standardized using customized acrylic stents that were grooved in the area of defect to provide reproducible insertion axis. Using the apical margin of the customized acrylic stent as the fixed reference point (FRP), the clinical measurements were made at the proximal line angle of the tooth with the associated bony defect at both test and control sites. Only one site representing the same deepest point of the defect was included. The following measurements were recorded at baseline and at all follow-up recall intervals.

- PPD = (FRP to BOP) – (FRP to gingival margin [GM])
- CAL = (FRP to BOP) – (FRP to cementoenamel junction [CEJ])
- BPD = (FRP to BPD) – (FRP to GM).

Conventional intraoral periapical radiographs (IOPARs) with X-ray mesh gauge (grid) were used. The radiographs were taken by one examiner throughout the period of the study with standardized parameters, exposure and processing. The paralleling technique was used with the conventional Kodak E-Speed IOPAR films of size 2. An X-ray mesh gauge was used along with the IOPAR films. The XCP-Rinn holder was used to provide projection standardization. Exposures were made at 70 kvp, 8 mA, and 2 mm of aluminum filtration. The exposure time was standardized as per the patient built. The focus to film distance was maintained at 16 inches.

The exposed films were processed with an automatic processor, which was set at standardized processing time and temperature. The films were processed in sets in fresh developer and fixer solutions were prepared as per the manufacturer's guidelines.

- The radiographic defect depth was calculated as the linear distance (in mm) measured from the alveolar crest to the base of the bone defect, i.e., (FRP to BOD) − (FRP to AC)
- Changes in ACL = (FRP to AC at baseline) − (FRP to AC at recall)
- ADF = Initial defect depth − defect depth at recalled time interval
- $$\text{PDF} = \frac{\text{Amount of defect fill}}{\text{Baseline defect depth}} \times 100$$

Preparation of citric acid-based nano hydroxyapatite composite graft

The experimental material was synthesized and characterized at the Department of Polymer Science, Sri Jayachamarajendra College of Engineering, Mysore - 570 006, Karnataka, India.

Material

NHA (Sybograft, particle size: 200–300 μ, Eucare pharmaceuticals (P) limited), citric acid monohydrate-ACS reagent, >99.0% (Anmol Chemicals, Mumbai), and 1,8 octanediol (Fluka) were used in this study.

Method

Preparation of poly (1,8-octanediol-co-citric acid)

Equimolar amounts of citric acid and 1,8-octanediol were added to a 250 ml three-neck, round-bottomed flask fitted with an inlet and outlet adapter. So, in the final product, each vial contained 7.7 g of material (5 g of NHA, 1.57 g of citric acid, and 1.05 g of 1,8 octanediol). The mixture was melted under a flow of nitrogen gas by stirring at 160°C–165°C, and then the temperature of the system was lowered to 140°C. The mixture was stirred for another hour at 140°C to create the prepolymer solution.[12]

The poly(1,8-octanediol-co-citric acid) (POC) prepolymer was mixed with various amounts of NHA particles to obtain composites of 65 wt% NHA by weight. In brief, POC prepolymer was mixed with the desired amount of NHA powder. The CA-NHA mixture was stirred until it became a uniform mass.[13]

This CA-NHA was placed in separate ten vials, and these vials were sent for gamma radiation to the Department of Studies in Physics, University of Mysore.

Sterilization

The samples were exposed to Co-60 gamma radiation using Gamma chamber (GC)-1200 under laboratory conditions at the Department of Studies in Physics, University of Mysore. The GC was calibrated with standard Co-60 source and the samples were kept at the middle of the sample chamber to get uniform dose. The dose rate of GC-1200 was 9.0 kGy/h and the samples were exposed in the total doses ranging from 25 to 27 kGy.[14,15]

Fourier transform infrared spectroscopy analysis

The characteristic bands for the presence around 568 correspond to (O-P-O) bending mode, whereas in the bone graft, a characteristic band of 601 cm^{-1}, this shift in the band is due to interaction between prepolymer and HA. The doublet in the range 1020 cm^{-1} was assigned to (P = O) antisymmetric stretching mode. These bands indicate the characteristic molecular structures of the polyhedrons of PO_{43-} in the apatite lattice. The characteristic peaks at 1722 cm^{-1} correspond to the C = O present in both HA and bone graft. Further, at 3426 cm^{-1}, the main hydroxyl vibration −OH was observed in HA, whereas in the HA-based bone graft, −OH band is shifted to 3458, this shift in the absorption band can be attributed to the interaction between oxygen of prepolymer and hydrogen atom of HA.

Thus, the molecular conformations were checked of NHA which remains unchanged in the citric acid-based NHA composite bone graft.

Surgical procedure

After following the aseptic protocol, the defect sites were selected randomly as test and control sites. The defect sites were anesthetized by 2% Lox with adrenaline (1:200,000) (Neon Antibiotics, Tarapur, Thane, India), using block and infiltration techniques.

Sulcular incisions were given on the facial and lingual sides using Bard-Parker® knife with blade no. 12. A full-thickness mucoperiosteal flap was raised to provide access to the defect. The defect was cleared of granulation tissue and thoroughly root planed using Gracey curettes (Hu-Friedy).

The surgical area was then irrigated with normal saline and carefully inspected for any remaining granulation tissue

or deposits. Any adherent granulation tissue was trimmed from the flaps.

The defects in the test site were filled with CA-NHA. The required quantity of composite graft was transferred from the vial to the Dappen dish and mixed with normal saline. When it became a cohesive mass, it was delivered into the vertical defects.

The defects in the control site were filled with NHA. The required quantity of graft was transferred from the vial to Dappen dish and mixed with normal saline. Flaps were repositioned and secured in place using sutures. Sling and interrupted sutures were placed using 4-0 Mersilk (Ethicon, Johnson and Johnson, Somerville, NJ, USA), given to obtain primary closure. The surgical sites were protected with a noneugenol periodontal dressing (Coe-Pak™) and analgesics were prescribed.

Postoperative instructions were given to all the patients and they were instructed to report back after 7 days.

At 7 days following surgeries, the dressings and sutures were removed and patients were inquired regarding discomfort, pain, and sensitivity.

Postoperative management

All patients received antibiotics for 5 days (3 × 500 mg amoxicillin) and painkillers for 3 days (Hifenac 2 × 100 mg). Postoperative care consisted of 0.2% Clohex rinses (Dr. Reddy's Lab, Hyderabad, India) twice a day for 1 week. After this period, patients were instructed to resume tooth brushing in the surgical area. The sutures were removed 7 days after the surgery. Recall appointments were scheduled for 3, 6, 9, and 12 months.

Statistical analysis

Statistical analysis was performed using the SPSS version 10.0 (SPSS Inc., IBM, Armonk, NY, USA).

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–Wilks test. If the assumption is not significant, then parametric test was carried out, otherwise nonparametric test was carried out. 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. In all the above tests, $P < 0.05$ was considered statistically significant.

Results

Ten patients (18 sites in test group and 15 sites in control group) completed the 12-month follow-up. Postoperative healing was uneventful and revealed good soft-tissue response to both treatments. No adverse complications were seen or reported during the surgical procedure or at subsequent postoperative appointments.

The participants of the study include four male and six female patients. The mean age of the patients was 38.10 ± 6.280 years [Table 1]. The type of defect and the number and distribution of sites are summarized in Table 2.

The full-mouth PI, GI, GBI, PPD, and CAL are summarized in Table 2. An improvement in all the above parameters was seen over a period of 12 months.

The values of site-specific PPD and CAL are summarized in Table 3.

The baseline PPD was 7.17 ± 1.200 mm in CA-NHA group and 7.47 ± 1.125 mm in NHA group [Table 3]. No statistically significant difference was found. At 12 months, the PPD was 3.72 ± 0.461 mm in CA-NHA group and 6.20 ± 0.862 mm in NHA group. Thus, the PPD decreased statistically significantly in both groups compared to the baseline data ($P < 0.001$). In addition, the test sites showed statistically significant improvement as compared to the control sites ($P < 0.001$).

The baseline CAL was 6.89 ± 0.676 mm in CA-NHA group and 6.93 ± 0.70 mm in NHA group [Table 3]. This difference was not statistically significant. At 12 months, the CAL was 3.83 ± 0.383 mm in CA-NHA group and 5.53 ± 0.743 mm in NHA group. In both groups, the CAL gain was statistically significant compared to baseline. In addition, the test sites showed statistically significant improvement as compared to the control sites ($P < 0.001$).

The baseline BPD was 8.00 ± 0.970 mm in CA-NHA group and 8.40 ± 1.056 mm in NHA group, with no statistically significant difference. At 12 months, BPD was 4.33 ± 0.686 mm in CA-NHA group and 6.73 ± 1.033 mm in NHA group. In both groups, the BPD decreased statistically significant compared to the baseline ($P < 0.001$). In addition, the test sites showed statistically significant improvement as compared to the control sites ($P < 0.001$).

In the radiographic parameters, at baseline, the mean ADF, PDF, and ACL were 0.00 in CA-NHA and NHA groups. At 12 months, in CA-NHA group, the mean ADF, PDF, and ACL were 2.00 ± 0.686 mm, 65.74 ± 27.602%, and 1.17 ± 0.857 mm, respectively, and in NHA group, at 12 months, the mean ADF, PDF, and ACL were 1.20 ± 0.775 mm ($P = 0.004$), 39.89 ± 24.432% ($P = 0.008$), and 0.60 ± 0.632 mm ($P = 0.042$), respectively. In both groups, the ADF, PDF, and ACL improved statistically significantly compared to the baseline ($P < 0.001$). In addition, the test sites showed statistically significant improvement as compared to the control sites at 12 months ($P < 0.001$).

Discussion

The results of the present study indicate that citric acid-based NHA or NHA alone can be used as a regenerative material

Table 1: Descriptive statistics of demographic, number of sites, test site, control site, maxillary and mandibular sites, 2-wall, 3-wall, and other defects

	Age	Number of site	Test site	Control site	Maxillary site	Mandibular site	2-wall defect	3-wall defect	Others
Mean±SD	38.10±6.280	3.30±1.567	1.80±1.135	1.50±0.707	1.90±1.287	1.40±1.265	1.40±0.0516	1.60±0.843	0.30±0.483
Minimum	30	2	1	1	0	0	1	1	0
Maximum	47	6	4	3	4	3	2	3	1
n	10	10	10	10	10	10	10	10	10

SD: Standard deviation

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

	Baseline	12 months	*P*
PI	2.42±0.418	0.42±0.063	<0.001
GI	2.39±0.404	0.54±0.107	<0.001
GBI	73.73±12.199	22.50±2.635	<0.001
PPD	7.60±0.843	3.80±0.632	<0.001
CAL	7.40±0.516	3.60±0.516	<0.001

Statistically significant at $P<0.05$. PI: Plaque index; GI: Gingival index; GBI: Gingival bleeding index; PPD: Pocket probing depth; CAL: Clinical attachment level

in intrabony defects in chronic periodontitis patients. Both the groups showed statistically significant improvement in the clinical as well as the radiographic parameters over a period of 12 months. In addition, statistically significant differences between the test sites as compared to the control sites were found in any of the investigated clinical and radiographic parameters 12 months after the therapy. A split-mouth design was chosen to facilitate the comparison of both CA-NHA and NHA groups under similar healing conditions by elimination of patient-specific variables.[16] The study duration of 12 months was chosen according to a study by Machtei in 1997,[17] which says that a 1-year end point should be established as the minimal interval for the measurement of clinical variables.

PPD, CAL, and BPD measurements were recorded with UNC 15 probe and were standardized using customized acrylic stents that were grooved in the area of defect, to provide a reproducible insertion axis. The apical edge of the custom-made acrylic stent was considered as FRP in the present study. Clark *et al*. in 1987[18] have reported that the measurements using a stent appear to be better than the measurements made using CEJ as the reference point. However, Watts in 1987[19] examined the possible sources of error with regard to probing measurement reliability with and without stent. He found that the stent made little difference to the overall reproducibility of probing depth, though it appears to reduce variation in different areas.

Full-mouth PI, GI, and GBI were assessed at baseline, 3, 6, 9, and 12 months. There was a statistically significant reduction in these parameters at 12 months, which indicate good oral hygiene maintenance by the patients.

Table 3: Site-specific pocket probing depth, clinical attachment level, bone probing depth, amount of defect fill, percentage defect fill, and alveolar crestal level

	CA-NHA	NHA	*P*
Clinical parameters			
PPD			
Baseline	7.17±1.200	7.47±1.125	0.468
12 months	3.72±0.461	6.20±0.862	<0.001
P	<0.001	0.001	
CAL			
Baseline	6.89±0.676	6.93±0.704	0.855
12 months	3.83±0.383	5.53±0.743	<0.001
P	<0.001	<0.001	
BPD			
Baseline	8.00±0.970	8.40±1.056	0.266
12 months	4.33±0.686	6.73±1.033	<0.001
P	<0.001	<0.001	
Radiographic parameters			
ADF			
Baseline	0.00	0.00	-
12 months	2.00±0.686	1.20±0.775	0.004
P	<0.001	<0.001	
PDF			
Baseline	0.00	0.00	-
12 months	65.74±27.602	39.89±24.432	0.008
P	<0.001	<0.001	
ACL			
Baseline	0.00	0.00	-
12 months	1.17±0.857	0.60±0.632	0.042
P	<0.001	0.054	

Statistically significant at $P<0.05$. CA-NHA: Citric acid-based nano hydroxyapatite composite graft; PPD: Pocket probing depth; CAL: Clinical attachment level; BPD: Bone probing depth; ADF: Amount of defect fill; PDF: Percentage defect fill; ACL: Alveolar crestal level

In the present study, the clinical parameters showed the following results at 12 months: the mean PPD reduction was found to be 3.45 ± 0.739 mm at test sites and 1.27 ± 0.263 mm at control sites. The mean gain in the CAL was found to be 3.06 ± 0.293 mm in test sites and 1.4 ± 0.039 mm in control sites. The mean reduction in BPD was found to be 3.67 ± 0.284 mm in test sites and 1.67 ± 0.023 mm in control sites. On intergroup comparison, the test sites showed statistically significant improvement in all the above-mentioned parameters as compared to the control sites ($P < 0.001$).

The radiographic parameters showed the following results at 12 months: the ADF was 2.00 ± 0.686 mm at test sites and 1.20 ± 0.775 mm at control sites. The PDF was 65.74 ± 27.602% at test sites and 39.89 ± 24.432% at control sites. The increase in the ACL was 1.17 ± 0.857 mm in test sites and 0.60 ± 0.632 mm at control sites. The ADF, PDF, and ACL showed statistically significant improvement in test sites as compared to the control sites ($P < 0.004$, $P = 0.008$, and $P = 0.042$, respectively).

To the best of our knowledge, no clinical study is published using citric acid NHA as a bone graft material in chronic periodontitis patients. No comparison of the efficacy of citric acid-based NHA and commercially available NHA is available. So far, only animal studies are available in orthopedics.

One such animal study was conducted by Sun *et al*.[3] which compared the efficacy of two different citric acid-based polymer HA composites; the first one being poly(1,8-octanediol citrate)-HA (POC-HA) and the second was cross-linked urethane-doped polyester-HA (CUPE-HA), as an alternative to autologous tissue grafts in the repair of skeletal defects. Radiological and histological data showed a significant enhancement of osteogenesis in defects in both the polymer groups as compared to the control groups at 1, 3, and 6 months posttrauma. These results show the potential of CUPE-HA and POC-HA bare implants as biocompatible, osteogenic, and off-the-shelf-available options in the repair of orthopedic defects. Based on this study, we have used POC-NHA composite graft in our study.

Guo *et al*.[20] conducted a study to evaluate the effect of biomimetic citrate-based POC-click-HA (POC-Click-HA) scaffolds in large segmental defects of bone. The results showed that all POC-Click-HA scaffolds exhibited good biocompatibility and extensive osteointegration with host bone tissue. Thus, it confirms the ability of scaffold to restore bone tissue and physiological functions in the early stages of recovery and the potential of citrate-based biomaterials in orthopedic applications.

Xie *et al*.[21] conducted a study to evaluate a novel injectable citrate-based mussel-inspired bioadhesive HA (iCMBA/HA) bone substitute for the treatment of comminuted bone fracture (CBF). *In vivo* evaluation of iCMBA/HA in a rabbit comminuted radial fracture model showed significantly increased bone formation, with markedly enhanced bending strength compared to the negative control. Neovascularization and bone ingrowth, as well as highly organized bone formation, were also observed, showing the potential of iCMBA/HA in treating CBF.

Tran *et al*.[4] conducted a study to evaluate the efficacy of citrate-based biodegradable composite in a rabbit lateral femoral condyle defect model and found that citrate-based polymer blend HA composite elicited minimal fibrous tissue encapsulation and was well integrated with the surrounding bone tissue.

Thus, the development of citrate-based biomaterials and their preliminary studies reveals the effects of free exogenous citrate on osteoblasts and shows the potential of citrate biomaterials to bridge the gap in HA crystals, thus proving the role of citrate molecules in regeneration, which has been previously overlooked.

NHA is one of the materials, which is used in bone regeneration, as it is a synthetic and osteoconductive material. The following studies show regenerative properties of NHA group.

Kasaj *et al*. in 2008[22] evaluated the efficacy of NHA paste in the treatment of intrabony defect in comparison with open flap debridement at 6-month follow-up and they found that the treatment of intrabony periodontal defects with NHA paste significantly improved clinical outcomes compared to open flap debridement. Jain *et al*.[23] conducted a 6-month split-mouth study and compared the nano-sized HA and β-TCP in the treatment of human intrabony defects. They concluded that NHA leads to significant improvement in early clinical and radiographical outcome as compared to β-TCP. Kasaj *et al*. in 2008[24] concluded the ability of NHA paste to promote human periodontal ligament cell proliferation and found that NHA paste was mechanically linked to activation of the epidermal growth factor receptor and downstream targets ERK1/2 and Akt, thus acting as a stimulator of cell proliferation of periodontal tissue regeneration.

According to Kim *et al*. in May 2000[25] who evaluated the limitations in measuring the clinical parameters in periodontal regenerative studies, it was shown that probing bone level most closely represented the actual bone level. They concluded that it might be a good clinical method for assessing the bone level following regenerative therapy in any site. We have measured the BPD and this could be considered the positive point for the study.

A limitation of clinical regeneration studies, such as the present one, is the inability to assess the histologic characteristics of the repaired tissues. Studies to examine the histologic nature of the interface of a treated bony defect and the root surface are difficult to conduct for ethical reasons.

The increase in the radiodensity in the defect, and hence a decrease in the defect size, signifies that the use of HA graft results in resolution of the intrabony defect. However, the nature of the regeneration, whether the graft acted as a filler material or allowed for ingrowth of the bone, cannot be inferred from the clinical and radiographic observations of the present study. Evaluation of the true nature of attachment requires histological investigation.

In addition, the present study has been concerned with clinical and radiographic observations using two different

graft materials and has been restricted to linear method of radiographic evaluation of defect fill. Future studies should incorporate precise radiographic evaluation using the advanced digital imaging facilities available.

Within the limitation of this study, the citric acid-based NHA composite graft showed a significant improvement in the clinical as well as the radiographic parameters over a period of 12 months. Thus, this graft can be considered a promising periodontal regenerative material.

Conclusion

To conclude, at 12 months, CA-NHA group showed statistically significant improvement in the clinical and radiographical parameters as compared to the NHA group. Thus, CA-NHA can be considered one of the newer materials in periodontal regeneration. A further study, which incorporates advanced digital imaging facilities and histological analysis, should be considered.

Clinical trial registration of India Registration number is CTRI/2016/11/007447.

Financial support and sponsorship

This research was carried out with the research grant awarded by the Indian Council of Medical Research, Department of Health Resource, Ministry of Health and Family Welfare, Ansari Nagar, New Delhi, for thesis research (Ref No. 3/2/October. 2015/PG-THESIS-HRD [24]). This aid provided was effectively used in this research work. I sincerely acknowledge their support.

Conflicts of interest

There are no conflicts of interest.

References

1. Newman MG, Takei HH, Klokkevold PR, Carranza FA. Clinical periodontology. In: Classification of Diseases and Conditions Affecting the Periodontium. 10th ed. St. Louis: Saunders; 2006. p. 103-4.
2. Dumitrescu AL. Chemicals in Surgical Periodontal Therapy. 1st ed. Berlin: Heidelberg, Springer-Verlag; 2011. p. 95-102.
3. Sun D, Chen Y, Tran RT, Xu S, Xie D, Jia C, *et al.* Citric acid-based hydroxyapatite composite scaffolds enhance calvarial regeneration. Sci Rep 2014;4:6912.
4. Tran RT, Wang L, Zhang C, Huang M, Tang W, Zhang C, *et al.* Synthesis and characterization of biomimetic citrate-based biodegradable composites. J Biomed Mater Res A 2014;102:2521-32.
5. Costello LC, Franklin RB, Reynolds MA, Chellaiah M. The important role of osteoblasts and citrate production in bone formation: "Osteoblast citration" as a new concept for an old relationship. Open Bone J 2012;4:27-34.
6. Hu YY, Rawal A, Schmidt-Rohr K. Strongly bound citrate stabilizes the apatite nanocrystals in bone. Proc Natl Acad Sci U S A 2010;107:22425-9.
7. Davies E, Müller KH, Wong WC, Pickard CJ, Reid DG, Skepper JN, *et al.* Citrate bridges between mineral platelets in bone. Proc Natl Acad Sci U S A 2014;111:E1354-63.
8. Okuda K, Tai H, Tanabe K, Suzuki H, Sato T, Kawase T, *et al.* Platelet-rich plasma combined with a porous hydroxyapatite graft for the treatment of intrabony periodontal defects in humans: A comparative controlled clinical study. J Periodontol 2005;76:890-8.
9. Silness J, Loe H. Periodontal disease in pregnancy. Ii. Correlation between oral hygiene and periodontal condition. Acta Odontol Scand 1964;22:121-35.
10. Loe H, Silness J. Periodontal disease in pregnancy. I. Prevalence and severity. Acta Odontol Scand 1963;21:533-51.
11. Ainamo J, Bay I. Problems and proposals for recording gingivitis and plaque. Int Dent J 1975;25:229-35.
12. Yang J, Webb AR, Ameer GA. Novel citric acid-based biodegradable elastomers for tissue engineering. Adv Mater 2004;16:511-6.
13. Qiu H, Yang J, Kodali P, Koh J, Ameer GA. A citric acid-based hydroxyapatite composite for orthopedic implants. Biomaterials 2006;27:5845-54.
14. Singh R, Singh D, Singh A. Radiation sterilization of tissue allografts: A review. World J Radiol 2016;8:355-69.
15. Adrovic F, editor. Kátia Aparecida da Silva Aquino. Sterilization by Gamma Irradiation, Gamma Radiation. InTech. 2012. ISBN: 978-953-51-0316-5, Available from: http://www.intechopen.com/books/gammaradiation/sterilization-by-gamma-irradiation.
16. Hujoel PP, Moulton LH. Evaluation of test statistics in split-mouth clinical trials. J Periodontal Res 1988;23:378-80.
17. Machtei EE. Outcome variables for the study of periodontal regeneration. Ann Periodontol 1997;2:229-39.
18. Clark DC, Chin Quee T, Bergeron MJ, Chan EC, Lautar-Lemay C, de Gruchy K, *et al.* Reliability of attachment level measurements using the cementoenamel junction and a plastic stent. J Periodontol 1987;58:115-8.
19. Watts T. Constant force probing with and without a stent in untreated periodontal disease: The clinical reproducibility problem and possible sources of error. J Clin Periodontol 1987;14:407-11.
20. Guo Y, Tran RT, Xie D, Wang Y, Nguyen DY, Gerhard E, *et al.* Citrate-based biphasic scaffolds for the repair of large segmental bone defects. J Biomed Mater Res A 2015;103:772-81.
21. Xie D, Guo J, Mehdizadeh M, Tran RT, Chen R, Sun D, *et al.* Development of injectable citrate-based bioadhesive bone implants. J Mater Chem B Mater Biol Med 2015;3:387-98.
22. Kasaj A, Willershausen B, Reichert C, Röhrig B, Smeets R, Schmidt M, *et al.* Ability of nanocrystalline hydroxyapatite paste to promote human periodontal ligament cell proliferation. J Oral Sci 2008;50:279-85.
23. Jain R, Kaur H, Jain S, Kapoor D, Nanda T, Jain M, *et al.* Comparison of nano-sized hydroxyapatite and β-tricalcium phosphate in the treatment of human periodontal intrabony defects. J Clin Diagn Res 2014;8:ZC74-8.
24. Kasaj A, Röhrig B, Zafiropoulos GG, Willershausen B. Clinical evaluation of nanocrystalline hydroxyapatite paste in the treatment of human periodontal bony defects – A randomized controlled clinical trial: 6-month results. J Periodontol 2008;79:394-400.
25. Kim HY, Yi SW, Choi SH, Kim CK. Bone probing measurement as a reliable evaluation of the bone level in periodontal defects. J Periodontol 2000;71:729-35.