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Research Article

A CLINICO-RADIOGRAPHIC EVALUATION OF HYDROXYAPATITE AND ß-TRICALCIUM PHOSPHATE WITH OR WITHOUT TYPE 1 COLLAGEN MEMBRANE IN THE TREATMENT OF INTRABONY DEFECTS IN MOLARS

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ABSTRACT

Background: The aim of the present study was to clinically and radiographically evaluate the efficacy of synthetic bonegraft in comparison with bone graft and GTR membrane in the treatment of intrabony defects in molars.

Materials and Methods: A total of 10 patients aged between 30 and 55 years diagnosed with moderate to advanced periodontitis with twenty bilateral angular defects were recruited for the study and were divided into two groups randomly (control group hydroxyapatite and ß-tricalcium **phosphate and test group:** hydroxyapatite and ß-tricalcium phosphate and type 1 collagen membrane. Clinical and radiographic parameters such as gingival index, plaque index, gingival bleeding index, pocket probing depth, relative attachment level (RAL), radiographic area of the defect, and the bone fill (BF) were recorded at baseline, 3 months, and 6 months.

Results and Conclusion: There was a significant reduction in probing depth and RAL from baseline to 3 and 6 months in both groups with statistically significant radiographic BF. Both the treatment modalities showed potential in enhancing periodontal regeneration.

INTRODUCTION

Periodontitis is an infectious disease of the periodontium and the changes occurring in the bone are crucial as the destruction of the bone is responsible for tooth loss.[1] The prime concern of any periodontal treatment is to have control over the errant microorganisms and resolution of soft tissue inflammation with the restoration of lost alveolar support. Resolution of soft tissue inflammation appears to be an established accomplishment after scaling, root planing, and oral hygiene instructions.[2] In advanced cases, the therapy includes surgical intervention, where the disease produces bony defects. Thus, further loss of connective tissue attachment could be prevented.[3] The two techniques with the most successful documentation of periodontal regeneration are osseous grafting and guided tissue regeneration (GTR).[4]

The application of bone replacement grafts would potentially manipulate the biological response into a regenerative rather than a predominantly reparative pattern which renders their use as an attractive choice in certain periodontal defect configurations. Bone grafts can be autogenous, allogenic, xenogenic, or alloplastic in nature.[5] Autogenous bone grafts were most popularly used for grafting, but the availability of the donor site and the limited quantity of the material caused limitations when these grafts were used. Allografts and xenografts, although, offered a solution to some of the above problems, the question of immunogenicity and disease transfer was often raised. Therefore, considering all the above-mentioned problems, alloplastic materials were introduced which are synthetic, biocompatible, inorganic bone graft substitutes, easily available, eliminate the need of a donor site, and carry no risk for disease transmission.[6]

The development of a two-phased calcium phosphate or biphasic calcium phosphate (BCP) ceramic made it possible to control the resorbability of the material and at the same time maintain its osteoconductive property. This ceramic is classified as resorbable because of the beta-tricalcium phosphate (β-TCP) content which resorbs much faster, whereas the presence of hydroxyapatite (HA) in the structure retards the resorbability of the material. BCP with >99% crystalline structure consists of 60% HA and 40% β-TCP in

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particulate form. Preclinical evidence suggests that the use of this ratio of HA and β-TCP allows better control of the bio-absorbable ability of the graft material resulting in accelerated new bone formation.[7,8] is a BCP consisting of HA and β-tricalcium phosphate in the weight (%percentage) ratio of approximately 70:30 and 60:40 that is biocompatible, nontoxic, resorbable, noninflammatory and bioactive. It causes no immunological, foreign-body, or irritating response, and has the excellent osteoconductive ability.[9]

The regeneration process of periodontium is initiated by periodontal ligament cells derived from the remaining periodontium as these cells are capable of differentiating into new cementoblasts, fibroblasts, and osteoblasts. For this, the barriers used are either nonresorbable or resorbable membranes. Among resorbable membranes, investigators have examined type-I collagen for the use in GTR procedures as it is a major extracellular macromolecule of the periodontal connective tissue, a weak immunogen and acts as a barrier for migrating epithelial cells. Furthermore, it is absorbable, thus, does not require a second surgical procedure for removal.[4] Type 1 collagen membraneis derived from a bovine source and is a resorbable, nonfriable barrier membrane obtained from type-l collagen derived from especially controlled and certified animals and is highly purified to avoid any antigenicity. Literature search revealed that less studies have been conducted using HA and β-tricalcium phosphate, 60:40) and Type 1 collagen membrane(type-1 collagen membrane). Hence, the purpose of the present study was to clinically and radiographically evaluate the efficacy of HA and β-tricalcium phosphate bone graft (60:40) in comparison with HA and β-tricalcium phosphate bone graft with type 1 collage GTR membrane in the treatment of intrabony defects in molars.

MATERIALS AND METHODS

A total of 10 patients aged between 30 and 55 years with a total of twenty defects in systemically healthy patients diagnosed with moderate to advanced periodontitis with bilateral clinical and radiographic evidence of angular defects were recruited for the study. Patients were selected from outpatient department of Periodontics Department of Govt Dental College Srinagar. Patients selected were divided into two groups randomly and treated according to split mouth design. Control group: 10 intrabony defects were subjected to open flap debridement with intrabony defect filled with Hydroxyapatite and ß-tricalcium phosphate bone graft material. Test group: 10 Intrabony defects were subjected to open flap debridement with intrabony defect filled with Hydroxyapatite and ß-tricalcium phosphate bone graft and Type 1 GTR membrane.

Inclusion Criteria

Patients presenting with chronic periodontitis (moderate to advanced periodontitis) with at least two periodontal osseous defects with bilateral vertical intrabony component in molars, free of systemic disease, age between 30 and 55 years and patients having probing depth of >5 mm.

Exclusion Criteria

Patients with compromised immune system, any history of recent periodontal surgery for the past 6 months, pregnancy and lactating females, smokers, history of antibiotic treatment for the past 6 months, uncooperative patients.Patients selected on the basis of above criteria were then explained about the treatment procedure and the associated risks and benefits. Written consent form was duly signed by the patients. Four weeks following phase I therapy a periodontal re-evaluation was performed to confirm the suitability of the sites for the study. The following recordings were made at baseline in the proforma designed for the study: Medical history, dental history, personal history, plaque index (PI),[10] gingival bleeding index (GBI),[11] probing pocket depth (in mm) measured by UNC-15 periodontal probe using gingival margin as a reference. Relative attachment level (RAL) (in mm) was recorded using acrylic stent on study cast for each patient and trimmed to height of contour of the teeth and one vertical groove prepared to reproduce the probe angulation and position. Digital radiography to assess the area of the defect was performed.[12] All parameters were recorded postoperatively at 3 and 6 months.

Surgical Procedure

Control Group

Patients were scheduled for periodontal surgery after phase-I therapy and preoperative measurements were recorded . After injecting local anesthesia (2% lignocaine with 1:80,000 adrenaline) at the site of surgery, sulcular incisions were given and full-thickness flaps were increased to retain sufficient tissue and to obtain primary closure. Intrabony defects were thoroughly debrided and root planed with hand and ultrasonic scalers. This was followed by condensation of Hydroxyapatite and ß-tricalcium phosphate bone graft material into the intrabony defect. Flaps were then repositioned and sutured. Periodontal dressing was applied over the site.

Test Group

The subjects after phase I therapy underwent periodontal flap surgery as mentioned above. After thorough debridement of intrabony defect, Hydroxyapatite and ß-tricalcium phosphate was condensed and covered by Type 1 collagen membrane bioabsorbable GTR membrane .The membrane was stabilized and then the flap was sutured . Periodontal dressing was applied over the site. Patients in both the surgical arms were subjected to a postoperative regimen of amoxicillin 500 mg (TDS) for 5 days and ibuprofen 400 mg TDS for 5 days and postoperative instructions were given. Patients were recalled for periodontal dressing and suture removal after 1 week. Patients were recalled at 3 and 6 months postoperatively and clinical parameters and radiographs were recorded at 3 and 6 months interval. Oral prophylaxis was performed in subjects if required at recall visits and oral hygiene instructions were reinforced.

Statistical Analysis

PI, GBI, probing depth (vertical), RAL and radiographic assessment for both the treatment groups recorded at baseline,

Table 1 Comparison of mean values of parameters at baseline, 3 and 6 months among the control and test groups

		Base line	3 month	6 month	Baseline to 3months(p)#	3-6 months (*P*)##	Baseline to 6 months (*P*)##
PI	Test	1.00±0.00	1.00±0.04	1.00±0.00	0.0470*	0.0470*	infinite
	Control	1.00±0.00	1.05±0.10	1.00±0.00	infinite	0.1229*	infinite
	Test versus control (*P*)#	0.1825	0.1824	Infinite			
GBI	Test	0.24±0.05	0.13±0.04	0.09±0.02	0.0005*	0.0011*	0.0000*
	Control	0.26±0.08	0.15±0.05	0.10±0.05	0.0005*	0.0002*	0.0000*
	Test versus control (*P*)#	0.4578	0.4134	0.3302			
PPD	Test	7.90±1.20	3.50±0.05	2.30±0.82	0.0000*	0.0019*	0.0000*
	Control	8.10±1.80	3.7±0.083	2.10±1.00	0.0000*	0.0086*	0.0000*
	Test versus control (*P*)#	0.7730	0.6653	0.6303			
RAL	Test	12.50±2.00	9.40±2.05	7.80±1.80	0.0003*	0.0066*	0.0001*
	Control	13.70±1.63	10.70±2.05	9.20±2.48	0.0058*	0.0224*	0.0003*
	Test versus control (*P*)#	0.1480	0.2260	0.1645			
Radiographic assessment	Test	138.60±70.4	26.64±21.7	7.24±6.5	0.0000*	0.0007*	0.0000*
	Control	123.45±46.3	28.75±16.7	11.04±7.90	0.0003*	0.0048*	0.0002*
	Test versus control (*P*)#	0.5791	0.8092	0.2556			

#Paired *t*-test, ##Repeated measures, *Significant difference ($P \leq 0.05$). GI: Gingival index, PI: Plaque index, GBI: Gingival bleeding index, PPD: Pocket probing depth, RAL: Relative attachment level

3 and 6 months were tabulated and subjected to statistical analyses. The statistical analyses were carried out using the SPSS Version 16.0. The mean, median, standard deviation, and percentage change of all the clinical parameters at baseline, 3 months, and 6 months were calculated for both test and the control groups. The various parameters measured as the continuous variables were compared between the test and control arm at baseline, 3 and 6 months using the paired *t*-test. The comparison of various parameters within the test and control groups at the various intervals was done using the unpaired *t*-test for the inter-interval comparison. The significance level was kept at 0.05 level.

RESULTS

During the course of the study, wound healing was uneventful. There were no postoperative complications in any patients, and none of the selected patients dropped out before the termination of the study. Periodontal assessment was conducted by single examiner to minimize the variations in the data [Tables 1 and 2].

Plaque Index

There was significant difference ($P < 0.05$) between baseline to 3 months in test group, whereas the difference was not significant ($P > 0.05$) from baseline to 3 months in the control group. No significant difference was found in the average scores of PI between baseline and 6 months.

Gingival Bleeding Index

Statistically significant differences ($P < 0.05$) were observed between different time intervals (e.g., 0–3 months, 3–6 months, and 0–6 months) in both test and control groups, whereas no significant difference ($P > 0.05$) was observed at baseline, 3 months, and 6 months between test and control group.

Probing Depth

The differences were statistically significant ($P < 0.05$), between different time intervals (e.g., 0–3 months, 3–6 months and 0–6 months) in test and control groups. No significant difference ($P > 0.05$) was present at baseline, 3 months, and 6 months between test and control group.

Relative Attachment Level

The differences were statistically significant ($P < 0.05$) between different time intervals (i.e., 0–3 months, 3–6 months, and 0–6 months) in test and control groups, whereas no significant difference ($P > 0.05$) was observed at baseline, 3 months, and 6 months between test and control groups.

Radiographic Area of Defect

In test group, the radiographic fill was increased to 80% between baseline to 3 months and to 94% from baseline to 6 months while in the control group, the radiographic fill was found to be increased to 76% at 3 months and 90% from baseline to 6 months [Graphs 2 and 3]. The differences were statistically significant ($P < 0.05$) in both test and control groups whereas no statistically significant mean differences in radiographic measurements were observed at baseline, 3 months, and 6 months ($P > 0.05$).

Parameter	Groups	Baseline	3 months	6 months
PI	Test	-1.6	1.5	0.0
	Control	-7.0	5.9	0.0
GBI	Test	42.31	30.35	61.37
	Control	39.80	31.03	59.19
PPD	Test	55.1	30.8	70.1
	Control	53.1	42.0	73.1
RAL	Test	23.8	14.5	35.9
	Control	22.0	14.5	33.0
Radiographic assessment	Test	0.00	0.80	0.94
	Control	0.00	0.72	0.90

GI: Gingival index, PI: Plaque index, GBI: Gingival bleeding index,
PPD: Pocket probing depth, RAL: Relative attachment level

DISCUSSION

The primary goal of periodontal treatment is the regeneration of lost attachment apparatus. Bone grafts, root surface biomodification and GTR techniques have been used to gain this therapeutic endpoint. BCP have presented significant advantages over other calcium phosphate ceramics. The stability of HA acts to maintain the augmented space, while ß-tricalcium phosphate promotes bone formation within that space. This controlled bioactivity and balance between

resorption/solubilization guarantees the stability of the biomaterial while promoting bone ingrowth.[13]

Furthermore, the particle size plays an important role in osteoconductivity of bone grafts. The size of particles of the test material in the current study ranged from 350 to 500 microns. In a re-entry study, it was observed that particles <300 µ undergo completely ionic dissolution and disappear by 1 year.[14] Green *et al*. have shown that pore diameters between 15 and 50 mm induce fibrovascular growth, whereas those between 50 and 150 mm stimulate osteoid formation.[15] Hence, the optimal particle size for alloplastic materials is 300–500 µm, a range that provided adequate inter-particular space for vascular invasion to occur. In this context, considerable research has been reported on "combined periodontal regenerative technique," that is, the combination of the epithelial-exclusion characteristics of membranes with the scaffold effect provided by bone grafts.[16,17]

Furthermore, these results were in accordance with that of Kaushick *et al*. who reported that PI values remained low throughout the study in both the groups which were not statistically significant.[18] In the control group, the observations recorded depict the reduction in GBI scores at baseline, 3 months, and 6 months as 0.26 ± 0.08, 0.15 ± 0.05, and 0.10 ± 0.05, respectively. A statistically significant difference ($P < 0.05$) was observed between different time intervals (e.g., 0–3 months, 3–6 months, and 0–6 months) in both test and control groups whereas no significant difference ($P > 0.05$) was observed at baseline, 3 months and 6 months between test and control group. Similar results were found in the earlier study done by Ratka-Kruger *et al*., Santosh Kumar *et al*.[4,19] Periodontal pocket is considered as the pathognomonic sign of periodontal disease and reduction in probing pocket depth is one of the requisites for successful periodontal therapy. The differences in mean values for probing depth were statistically significant ($P < 0.05$), between different time intervals (e.g., 0–3 months, 3–6 months, and 0–6 months) in test and control groups when intragroup comparison was performed using paired t-test, whereas no significant difference ($P > 0.05$) was observed at baseline, 3 months, and 6 months between test and control groups, respectively. These results were in accordance with the results of the study done by Debnath *et al*., Mopur *et al*., Lee *et al*., Sowmya *et al*.[20-23] The results of this study were in contrary to the results observed by Saini *et al*.[24] RAL has been widely accepted as the primary clinical endpoint of regenerative attempts around natural teeth. Significant loss in clinical attachment levels is reflected in histological loss of the tooth's attachment apparatus.[25] The differences in the mean RAL measurements were statistically significant ($P < 0.05$) between different time intervals (i.e., 0–3 months, 3–6 months, and 0–6 months) in test and control groups while no significant difference ($P > 0.05$) was observed at baseline, 3 months, and 6 months between test and control groups. Similar results were observed by Sowmya *et al*.,[23] Shetty and Bose.[26] In the present study, Hydroxyapatite and ß-tricalcium phosphate was used in ratio of 60:40 for intrabony defects. A study conducted by Chandrashekar *et al*., determined the efficacy of Hydroxyapatite and ß-tricalcium phosphate (70% HA + 30% ß -TCP) as a bone graft material in the treatment of vertical defects in the generalized chronic periodontitis patients and found a significant reduction in probing pocket depth and gain in clinical attachment level.[9] In the study by Debnath *et al*., it has been identified that good bone defect fill, evidenced radiographically, occurs with the use of HABG Active" in the treatment of periodontal infrabony defects.[20] The improvement in the clinical probing and RAL was well supported by the decrease in the radiographic area of the intrabony defect in both test and control groups of the present study, which was determined in a manner similar to that as described by Eickholz *et al*.[12] Radiographic monitoring of alveolar bone changes following regenerative procedures is a noninvasive, painless alternative to direct bone measurements; regeneration in periodontal defects is usually measured by bone fill (BF) in angular defects.[23] Digitized imaging has become an important tool in determining the subtle alterations seen on images of bone defects, not only because of the magnification of the image on the screen but also due to the possibility of adjusting its brilliance and contrast.[17] The differences control groups.

However, on inter-group comparison of the mean difference in radiographic measurements at baseline, 3 months, and 6 months, scores were found to be statistically not significant ($P > 0.05$). These results were in accordance to the studies conducted by Chandrashekar *et al*., Debnath *et al*., Shetty and Bose[9,20,26] Bansal *et al*. evaluated the depth of intrabony defect using the same landmarks used in the current study after 6 months and reported significant gain in the bone height and reduction in the depth of the defect.[27] Similar results were obtained in the studies conducted by Santosh Kumar *et al*. at the end of 9 months.[4] BF is a desirable result of periodontal regeneration procedures. Sowmya *et al*. demonstrated radiographically the BF in test and control groups. On intergroup comparison, the difference being statistically significant ($P < 0.01$) in favor of test group.[23] Hashimoto-Uoshima *et al*. in their histologic study found that BCP (80% ß -TCP/20% HA) has osteoconductive potential and this potential may be related to degradation by macrophage phagocytosis.[27] The main feature of BCP ceramic is their ability to form a strong direct bond with the host bone, resulting in a strong interface, compared to bioinert or biotolerant materials which form a fibrous interface. Ellegaard and Loe (1971), in their study on 191 defects, found that complete regeneration had occurred in 70% of three-walled defects and 45% of two-walled defects.[17] This confirms to the prevalent opinion of clinicians about the number of walls of bony defects that is required to have a better degree of BF. Intrabony 3 wall defects provide a good scaffold as they remain surrounded by bone from three sides and tooth from the fourth, thus providing a good stability to the graft material. Therefore, it could be concluded that the defect configuration plays an important role in the outcome bone graft as the biomechanics of regeneration.[28] The bone graft material acts as a defect filler, supporting the overlying GTR membrane and avoiding the membrane collapse. It also acts as a framework into which bone-forming cells and blood vessels integrate leading to the formation of healthy new bone and subsequent rapier of the bone defect. Biograft bone regenerative materials are both

biocompatible and bioactive and HA phase is the major constituent.[9] Collagen membranes are ideal resorbable GTR membranes. It is believed that bone grafts not only provide osteoinductive/osteoconductive capacity for regeneration, but also maintain the space. Jain and Deepa in their study concluded that the combination of freeze‐dried bone allograft with bioabsorbable GTR membrane Healiguide® demonstrated a significant improvement in the probing depth and RAL at 6 months postsurgery in the treatment of Grade II furcation defects.[29] A study comparing the use of type 1 collagen and expanded polytetrafluoroethylene (ePTFE) in treating human mandibular Class II furcations reported that the collagen membrane evoked a lower inflammatory response than did the ePTFE, the material is pliable when moist and conforms well to the surgical area, collagen provides a thrombogenic surface that is sealed coronally to the root surface by a fibrin clot and there was no allergic response to the collagen.[30] The use of a membrane stabilizes the wound and protects the root surface‐adhering fibrin clot from tensile forces acting on the wound margin. Being chemotactic for fibroblasts, the collagen membrane barrier may act to enhance and protect the initial clot formation onto the root surface by acting as a scaffold for cell adhesion and ingrowth aiding in the formation of new attachment and regeneration during GTR procedures.[31] Furthermore, the results of the study conducted by Oh *et al*. 2016, showed that both HA and beta‐tricalcium phosphate showed satisfactory gap healing without complications and could be successfully used as alternative healing materials in opening wedge high tibial osteotomy. Further this study also showed that beta‐tricalcium phosphate has superior absorbability than HA and osteoconductivity showed no significant difference.[32] To the best of our knowledge, no human study was found in the course of the current study which evaluated the use of HA + ß‐TCP in the ratio of 60:40 and Type 1 collagen membrane GTR membrane in the treatment of periodontal intrabony defects.

CONCLUSION

Within the limitations of this study, it could be concluded that both the treatment modalities showed favorable clinical and radiographic improvements from baseline to 3 and 6 months. However, there was no statistically significant difference in the scores when compared between the test and the control groups. Results suggested that there was an additional benefit with the combination of Hydroxyapatite and ß‐tricalcium phosphate bone graft and Type 1 collagen GTR membrane in intrabony defects although not statistically significant when compared to Hydroxyapatite and ß‐tricalcium phosphate alone. This combination treatment modality provided improved outcomes in terms of clinical.

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