Biomaterials in periodontal osseous defects

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ABSTRACT

Introduction: Osseous defects in periodontal diseases require osseous grafts and guided tissue regeneration (GTR) using barrier membranes. The present study was undertaken with the objectives to clinically evaluate the osteogenic potential of hydroxyapatite (HA), cissus quadrangularis (CQ), and oxidized cellulose membrane (OCM) and compare with normal bone healing.

Materials and Methods: Twenty subjects with periodontitis in the age group ranging from 20 years to 40 years were selected from our outpatient department on the basis of presence of deep periodontal pockets, clinical probing depth ≥ 5 mm, vertical osseous defects obvious on radiograph and two- or three-walled involvement seen on surgical exposure. Infrabony defects were randomly divided into four groups on the basis of treatment to be executed, such that each group comprised 5 defects. Group I was control, II received HA, III received CQ and IV received OCM. Probing depth and attachment level were measured at regular months after surgery. Defects were re-exposed using crevicular incisions at 6 months.

Results: There was gradual reduction in the mean probing pocket depth in all groups, but highly significant in the site treated with HA. Gain in attachment level was higher in sites treated with HA, 3.2 mm at 6 months.

Conclusion: Hydroxyapatite and OCM showed good reduction in pocket depth, attachment level gain and osseous defect fill. Further study should be conducted by using a combination of HA and OCM in periodontal osseous defects with growth factors and stem cells.

Keywords: *Cissus quadrangularis, hydroxyapatite, oxidized cellulose, periodontal osseous defects.*

INTRODUCTION

Periodontal diseases lead to alterations in the morphological features of the bone resulting in osseous defects. Currently, the materials used for periodontal tissue regeneration include various osseous grafts and guided tissue regeneration (GTR) using barrier membranes. Over the past several years, hydroxyapatite (HA) and related calcium phosphate are being used as bone substitutes as their crystalline and chemical compositions are closely allied to the mineral components of the bone.¹ Use of traditional medicinal herbs can be regarded as a good alternative for synthetic materials in bone regenerative procedures. *Cissus quadrangularis* (CQ) is an indigenous plant known to possess accelerating effect on the repair of bone fracture and stabilization of implants in the long bones of rabbit.² The plant extract contains appreciable quantity of highly potent anabolic steroids, vitamins C and A.

Its components are believed to be useful in not only building up of chemical composition of the bone but also in bringing about its functional efficiency.²

After periodontal therapy, the epithelium from the flap margin has a strong tendency to migrate apically along the root surface.³ In order to prevent the apical migration of the epithelium and promote regeneration of periodontal tissue, GTR has been described using barrier membranes under surgical flap to prevent epithelial in-growth into healing wound. Although, Millipore filter⁴ and polytetrafluoroethylene (PTFE) membrane have been shown to be successful, these materials are not biodegradable and therefore require a second surgical procedure for the removal of the membrane once healing has occurred. Oxidized cellulose membrane (OCM), commercially available as resorbable hemostatic dressing, has also been demonstrated to possess antibacterial properties⁵ and can be used as GTR membrane.

This study was undertaken with the objectives to clinically evaluate the osteogenic potential of HA, CQ and OCM, and compare with normal bone healing.

MATERIALS AND METHODS

Twenty subjects with adult periodontitis in the age group ranging from 20 years to 40 years were selected from our outpatient department on the basis of the presence of deep periodontal pockets where clinical probing depth was ≥ 5 mm, and vertical osseous defects were seen on the radiograph and surgical exposure showed two or three walls involved. Subjects with any systemic complications, allergies, acute oral afflictions, mucogingival problems, teeth with furcation involvements, and any dental pathology were excluded. A general assessment of the selected subjects was made through their systemic and dental history, clinical examinations, and routine investigations. Written informed consent was obtained and full-mouth intra-oral periapical radiographs were recorded. Study was approved by the institutional research committee. All subjects were treated with initial phase of therapy involving scaling, root planning, oral hygiene instructions, and occlusal adjustment if occlusal trauma was present.

An acrylic stent⁶ was fabricated and grooved at the site of the defect in an occlusoapical direction on the facial surface of the stent, to allow repeated placement of the probe in the same direction at different measuring times. All measurements were made by the same operator for pre-operative measurements like probing depth and attachment level. The probing pocket depth was measured from the gingival margin to the base of the pocket. The attachment level was measured from the cemento-enamel junction (CEJ) to the base of the pocket. If gingival margin was coronal to the CEJ, then the attachment level was measured by subtracting the distance from the gingival margin to the CEJ from the pocket depth. When the gingival margin coincided with CEJ, the loss of attachment was equal to the pocket depth. When the gingival margin was located apical to CEJ, loss of attachment was greater than the pocket depth and therefore the distance between CEJ and gingival margin has been added to the pocket depth.

Under local anesthesia, a full thickness flap was reflected using crevicular incision to conserve as much of the soft tissue flap as possible and ensure adequate coverage of the grafted site. Granulation tissue was curetted and the exposed root surfaces were thoroughly planed to a smooth hard surface. The flap was debrided of inflammatory tissues and epithelial strands from the inner surface.

The architecture of the defect was observed radiographically and recorded. Only two- and three-walled osseous defects were considered. Infrabony defects were randomly divided into groups on the basis of treatment to be executed, such that each group comprised five defects:

Group I—Control group were treated by debridement alone. Flaps were closed with interrupted interdental suture after the debridement.

Group II—Received HA in the osseous defect after debridement (*OsteoGen*[®], Implants Ltd.). Hydroxyapatite crystals were mixed with autologous blood to make a thick paste and was filled in the osseous defect up to the alveolar crest and sutured.

Group III—Received CQ osseous defect after debridement. Fresh stem of the plant was washed in normal saline, cut into small pieces, made into a thick paste, and put into a sterile test tube with the mouth closed. The material was placed into the defect in successive increments, condensed up to the level of the alveolar crest and the flaps were sutured.

Group IV—Received oxidized regenerated cellulose membrane (*Surgicel*[®], Ethicon) to cover the osseous defect after debridement and the wound was closed.

Postoperatively, the stent was placed on to the teeth and measurements were made from the CEJ to the base of the defect, CEJ to the alveolar crest, and alveolar crest to the base of the defect. The sutured surgical area was covered with a periodontal pack (Coe-Pack), chlorhexidine mouthwash was advised twice daily and they were recalled after 1 week for checkup, removal of sutures, and periodontal pack. They were thereafter recalled every month for brushing, scaling, and root planning. Probing depth and attachment level were measured at 1, 3, and 6 months after surgery. Defects were re-exposed using crevicular incisions at 6 months. Following removal of all soft tissues from the defect, the stent previously fabricated was placed on the tooth surface to record measurements from the CEJ to the base of the defect, from CEJ to the alveolar crest, and from the alveolar crest to the base of the defect. The site was then sutured with plain interrupted sutures.

RESULTS

Soft tissue measurements comprising probing pocket depth are presented in Table 1. There was gradual reduction in the mean probing pocket depth in all groups, but highly significant in the site treated with HA. The gain in the attachment level had almost the same pattern as in the probing pocket depth (Table 2) as was higher in the sites treated with HA. The maximum gain in the attachment level was 3.2 mm at

Table 1 Comparison of probing pocket depth reduction with pre-operative probing pocket depth at different intervals from group I to group IV

Time (mo)	Group I			Group II			Group III			Group IV		
	Mean (SD)	t	P	Mean (SD)	t	P	Mean (SD)	t	P	Mean (SD)	t	P
1	1.4 (0.548)	5.715	<0.01	2.4 (0.547)	9.810	<0.001	1.8 (0.836)	4.810	<0.01	2.4 (0.894)	5.999	<0.01
3	1.6 (0.547)	6.531	<0.01	3.2 (0.837)	8.548	<0.01	2.2 (0.836)	5.879	<0.01	3.4 (0.547)	13.880	<0.001
6	2.4 (1.14)	4.706	<0.01	3.4 (0.548)	13.872	<0.001	2.8 (0.447)	13.99	<0.001	3.8 (0.836)	10.155	<0.001

SD=standard deviation.

Table 2 Comparison of attachment level gain with pre-operative attachment level at different intervals in various groups

Time (mo)	Group I			Group II			Group III			Group IV		
	Mean (SD)	t	P	Mean (SD)	t	P	Mean (SD)	t	P	Mean (SD)	t	P
1	1.0±0.707	3.162	<0.05	1.6±0.548	6.531	<0.01	1.2±0.447	5.999	<0.01	2.4±1.140	4.706	<0.01
3	1.0±0.707	3.162	<0.05	2.2±1.304	3.772	<0.05	1.2±0.836	3.207	<0.05	2.6±0.547	16.427	<0.001
6	1.4±0.548	5.715	<0.01	3.2±1.304	5.487	<0.01	1.6±0.547	6.531	<0.01	2.6±0.547	10.614	<0.001

SD=standard deviation.

Table 3 Intergroup comparison of pocket depth reduction and attachment level gain

Groups	Probing pocket depth reduction						Attachment level gain					
	1 month		3 months		6 months		1 month		3 months		6 months	
	t	P	t	P	t	P	t	P	t	P	t	P
I vs II	2.888	<0.05	3.577	<0.01	1.768	NS	1.5000	NS	1.809	NS	2.846	<0.05
I vs III	0.894	NS	1.341	NS	0.730	NS	0.534	NS	0.408	NS	0.577	NS
I vs IV	2.133	NS	5.2	<0.001	2.557	<0.05	2.332	<0.05	4.001	<0.01	3.467	<0.01
II vs III	1.342	NS	1.890	NS	1.897	<0.05	1.265	NS	1.443	NS	2.530	<0.05
II vs IV	0	NS	2.236	NS	0.894	NS	1.415	NS	0.632	NS	0.948	NS
III vs IV	1.094	NS	2.683	<0.05	2.358	<0.05	2.192	NS	3.130	<0.01	2.888	<0.05

6 months in this group. In sites treated with OCM, the attachment level gain was highly significant ($P<0.001$) at 3 and 6 months with 2.6 mm gain at 6 months. In group III, the attachment level gain was almost similar to that in control group. Intergroup comparison (Tables 3 and 4) showed non-significant difference between group II and IV.

DISCUSSION

Regeneration of the periodontium destroyed by the inflammatory periodontal diseases has been an elusive goal sought by all who treat periodontal problems. Currently, there is no procedure that results in a predictable regeneration of the

bone, periodontal ligament, and cementum, when significant alveolar bone destruction has occurred.⁷ Autografts have been the most widely used graft materials in the periodontal osseous defects. These autografts are not only unpredictable, but the paucity of suitable donor tissue has also been deterrent to its use. Allografts and xenografts have also been tried but no substantial evidence of success has been evident. Currently, emphasis has been placed on the utilization of synthetic graft materials for bone regeneration. One of the first synthetic graft materials used in periodontics was plaster of paris but reports showed that the materials had no osteogenic potential. Calcium phosphate biomaterials such as HA and tricalcium phosphate have shown to have good osteoconductive potential. Coralline-based non-ceramic form of HA belongs to porites group and has an architecture

Table 4 Intergroup comparison of hard tissue measurements

Groups	Mean osseous defect fill at 6 months re-entry		Percentage fill of original defect at 6 months		Percentage change in alveolar crest height at 6 months re-entry	
	<i>t</i>	<i>P</i>	<i>t</i>	<i>P</i>	<i>t</i>	<i>P</i>
I vs II	6.002	<0.001	4.968	<0.01	2.789	<0.05
I vs III	1.706	NS	1.440	NS	6.534	NS
I vs IV	6.002	<0.001	5.772	<0.001	2.277	NS
II vs III	5.099	<0.001	2.958	<0.05	2.400	<0.05
II vs IV	0	NS	0.1103	NS	0.725	NS
III vs IV	5.099	<0.001	3.179	<0.05	1.829	NS

very similar to that of spongy bone with interconnecting pores of diameter 100–200 µm. It is biocompatible and its gradual resorption is accompanied by bone formation.⁸ Use of demineralized freeze-dried bone allograft overcomes many of the problems associated with autografts, but these materials are human products procured from banks; hence, there is a small but measurable risk of disease transmission estimated at one in 1 million to one in 8 millions.⁹

Among various herbal medicaments, the paste and extract of CQ has been used for its potential as a bone regenerative material. Udupa et al¹⁰ conducted a study by applying plant extract on the fractured bones of dogs and found a marked difference in the progress of endochondral ossification. The microscopic examination of the healed area showed an excessive multiplication of chondroblasts and osteoblasts encroaching the area of collagen fibers. Saxena et al studied the positive effect of CQ on the union of fractured bony fragment of the mandible.

Guided tissue regeneration using non-resorbable membranes has been used successfully but because of additional surgical procedure required to remove the material and it being more expensive, a comparatively cheaper and bioresorbable, OCM has been tried in this study.

Healing and attachment requires an interaction between fibroblasts and the root surfaces. The root surfaces in a diseased environment undergo certain changes which inhibit the close adherence of fibroblasts to the root surface. The efficacy of root planning has been well documented in making the root surface disease-free and histocompatible for the surrounding soft tissue.¹¹ Accordingly, a meticulous root planning was performed in all patients.

Pocket depth and attachment level recording were carried out at 1 month postoperatively, as dentoepithelial junction establishes by 3 weeks in a human.¹² The re-entry surgery was performed after 6 months to evaluate the regeneration of the bone in the defect site. Various investigators have noticed no further tissue alteration and periodontal tissue regeneration after 6 months of periodontal surgery.¹³ Langer,¹⁴

on the other hand, discussed the rationale for the early re-entry procedure of autogenous bone grafts after 3 months as he observed no particular advantage of waiting for a longer period to access the result of an implant procedure.

Hydroxyapatite was well tolerated and did not seem to evoke any apparent adverse inflammatory response. The re-entry data coincided with the clinical data. Thus, a measurable improvement in the gain in the attachment level was duplicated with an equivalent improvement in the depth of osseous defect seen at re-entry. At re-entry, the surface of the grafted area appeared pebbly and hard on light touch and resisted penetration of the probe. This was in accordance with the study of Rabalais et al.¹⁵ The bone formation at the site grafted by HA was due to gradual resorption accompanied by simultaneous bone formation. Results demonstrated a very definite advantage of bone regenerative materials when compared with control.

In this study, the maximum fill of osseous defect was seen in those treated by HA (3 mm) which was in accordance with (3.36 mm) that of Meffert et al.¹⁶ The mean osseous defect fill of 0.60 mm was seen in control sites while it was 1.6 mm in the sites treated with CQ. High contents of vitamins A and C, calcium oxalate, and some unknown anabolic steroids may be responsible for better osseous fill in CQ sites as compared with control sites.¹⁷ The mean osseous fill in group IV was 3 mm at 6 months re-entry. This result coincided with the result of Mattson et al who reported 3.5 mm osseous fill treated by collagen barrier membrane.¹⁸

The percentage of defect resolution was 48.66% in sites treated by HA and 23% in the control. These results are in agreement with the result of Meffert et al¹⁶ with 53.57% in HA-treated site and 19.49% in the control. Rabalais et al¹⁵ showed 48.5% resolution in HA-treated sites.

The percentage fill of original defect is 61% in HA-treated sites, and 14% in the control sites. Meffert et al¹⁶ observed 66.89% and 9.91% fill of original defect in HA experimental and control group, respectively. The percentage change in the alveolar crest height was 9% reduction in

the control site while 12.33% increased alveolar crest height was observed in HA-treated sites.¹⁵ However, most of the workers have noted the loss of crestal bone height.

The percentage of original defect resolved in CQ was 33.72% while 28.72% fill of original defect and 5% reduction in the crestal bone height were also noted. The bone fill in the site treated with CQ may have been primarily due to the stimulation of the cells of mesenchymal origin such as fibroblasts, chondroblasts, and osteoblasts.¹⁶

In the sites treated with OCM, the percentage original defect resolved was 52.67% and the percentage fill of the original defect was 60%, in accordance with the findings of Mattson et al who reported the mean percentage osseous defect fill of 61%. The crestal bone height gain was noted as 7.3%.

Sites treated with HA and OCM in our study showed the same amount of defect fill of 3 mm. In the terms of percentage original defect resolved, OCM showed better results as compared with HA. The percentage original defect fill was almost the same in both group IV and group II as 60% and 61%, respectively.

It was observed that at the time of placement of HA in the osseous defects, some material gets spilt out of the grafted site. The use of OCM along with HA prevents the spill of HA. Oxidized cellulose membrane acts as a barrier membrane as well as a hemostatic material and when it comes in contact with blood, it forms a clot and helps in the regeneration of bone.¹⁹

CONCLUSION

Hydroxyapatite and OCM showed similar reduction in pocket depth, attachment level gain, and osseous defect fill. *Cissus quadrangularis* showed better osseous healing than the control group. Further study should be conducted by using a combination of HA and OCM in periodontal osseous defects with growth factors and stem cells.

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