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CASE REPORT

Comparison of amnion membrane and bone graft with bone graft alone in the treatment of periodontal intrabony defects: A case study

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ABSTRACT

Background: Combination guided tissue regeneration for the treatment of periodontal intrabony defects is an accepted treatment modality for many years and has undergone a number of changes. Human amniotic membrane allografts have been used for a variety of reconstructive surgical procedures since the early 1900s. The purpose of this study was to clinically and radiographically evaluate the efficacy of amniotic membrane as a guided tissue regenerative material in the treatment of human periodontal intrabony defects.

Method: Thirty six intrabony defects in eighteen systemically healthy subjects having moderate to severe chronic periodontitis were randomly assigned to control group (treatment with hydroxyapatite bone graft) and test group(treatment with hydroxyapatite and amnion membrane). The plaque index, gingival index, probing pocket depth (PPD), clinical attachment level (CAL), and gingival recession (REC) were recorded at baseline, and were reevaluated at 3 and 6 months. In addition to this, radiographic bone fill was assessed using radiovisiograph. At the test site, hydroxyapatite bone graft and amnion membrane was placed, whereas at the control site, only hydroxyapatite bone graft was placed. Clinical and radiographic evaluations were made at baseline, at 3 and 6 months following surgery.

Results: In the control group, the mean reduction of PPD was 3.11 mm and the mean CAL gain was 3.78 mm. In the test group, the mean PPD reduction was 4.33 mm and mean gain in CAL was 5.55 mm after 6 months which was statistically significant. The mean gain in radiographic defect fill was 53.87% in the test and 50.76% in the control group after 6 months which was statistically significant. A significant decrease in mobility and gingival index was observed. .

Conclusion: Amnion membrane is an effective treatment option for the reconstruction of periodontal intrabony defects as it led to statistically significant improvements in the clinical and radiographic parameters.

Key words: Periodontitis; intrabony defects; regeneration

INTRODUCTION

Periodontitis involves an inflammatory process of multi-factorial origin, affecting the periodontal tissues viz alveolar bone, periodontal ligament, cementum of the tooth and gingiva, and provoking the destruction of the supporting tissues to the teeth. The ultimate goal of periodontal therapy is to regenerate the lost periodontal tissues caused by periodontitis. Historically, regenerative treatment for periodontal intrabony lesions has focused on the use of monotherapies to restore the lost hard tissues: bone, cementum and a functional periodontal ligament.[1] Combination regenerative approaches have been quite successful for treating the more challenging intrabony lesions, particularly as the size and complexity of the lesion increases. Typically, these efforts have included a bone replacement graft that has been covered by an exclusionary barrier. Although this approach to regeneration has been quite successful, there still remains a concern that the graft material may act strictly as a space filler and not have the intrinsic biologic capability to provide for regeneration.[2]

Combined regenerative approaches for teeth with intrabony or furcation lesions have included membranes to prevent apical migration of both the epithelial cells and connective tissue into the space, to facilitate containment of the bone replacement graft along with stabilizing the newly formed clot. A resorbable amnion chorion membrane,continues the progressive development of GTR as it is the first membrane to provide growth factors and cell adhesion proteins within the membrane.

Human amnion membrane has been used as a biomaterial for surgical reconstruction for almost 100 years. There has been increasing interest in studying the biology of amnion membrane because it exhibits tremendous potential for therapeutic purposes due to its absorption, high biocompatibility, regenerative capacity and ease of implementation.[3B] As it contains various growth factors, it is speculated to be useful in regeneration of osseous defects in periodontal surgeries. Specifically the membrane has gained importance because of its ability to reduce scarring and inflammation; enhance wound healing; and serves as a scaffold for cell proliferation and differentiation and in its antimicrobial properties.

It is a composite membrane consisting of pluripotent stem cells, possessing the ability of transdifferentiation to other cellular elements of the periodontium, thus makes it a suitable material for guided tissue regeneration.[3A] Important to the success of any allograft is its safety and biocompatibility. Amnion contains several extracellular matrix (ECM) proteins, including fibronectin, laminins and collagen types I, III, IV, V, and VI. These ECM proteins play an important role in the biocompatibility of amnion tissue.[4] The nature of the biocompatibility may be attributed, in part, to the the presence of growth factors and TIMP-1 in the membrane. Laminin-5 played a strong role in the adhesion between the tooth surface and the directly attached to the tooth-facing cells (DAT cells) of the junctional epithelium.[5] The attachment of DAT cells forms via hemidesmosomes where both laminin-5 and the α6β4 integrin play critical roles.[6]

Ceramics are the recently used alloplastic materials, they can be either bioactive or bioinert. Bioactive ceramics include hydroxyapatite. Hydroxyapatite is an apatite of calcium phosphate $Ca_{10}(PO_4)_6(OH)_2$, a ceramic naturally found in vertebrate tooth and bone. The compound has a Ca/P mole ratio of 1.67 and is formed by precipitation of calcium nitrate and ammonium dihydrogen phosphate. It is a biocompatible, nontoxic, slowly resorbing, osteoconductive, osteophillic material and has close structural and chemical resemblance to bone mineral, but not identical.

Hence, the purpose of the present study was to assess the regenerative capacity of hydroxyapatite bone graft in conjunction with bioactive amnion membrane in periodontal intrabony defects.

MATERIALS AND METHOD

STUDY METHOD

Patient Selection

18 systemically healthy subjects (10 males and 8 females) suffering from moderate to severe chronic periodontitis, age between 30-65 years and probing pocket depth of 6mm or more at the experimental site were enrolled.

Patients who had any medical condition or were on therapeutic regimen that could decrease the probability of soft tissue or bone healing, pregnant or lactating women, teeth with furcation involvement, non vital or endodontically treated teeth, third molars, one walled defects, patients with parafunctional habits i.e. Bruxism and who had periodontal surgery in last 6 months, allergic to tetracycline, chlorhexidine were excluded.

The study was approved by the institutional ethical committee.

STUDY MATERIAL

Commercially available thin amnion membrane averaging 300µm in cross sectional thickness and Hydroxyapatite bone graft (Biograft ® - HA ^{NANO})were used as the material for study.

Amnion membrane requires no rehydration prior to placement and self-adheres to the surgically prepared, exposed tooth root and adjacent bone, eliminating the need for internal sutures. Before the membrane is applied, the intrabony defect should be prepared after thorough removal of granulation tissue. Membrane is applied with rough (chorionic) surface next to the defect. Care is taken to ensure that there are no air bubbles trapped between the membrane and defect. Once the membrane is placed, gingival tissue is advanced and sutured into place, fully covering the membrane.

CLINICAL PARAMETERS

The graft material was assessed on the basis of evaluation of certain selected clinical parameters, soft and hard tissue measurements of the experimental defect. The clinical parameters i.e Probing pocket depth, Relative attachment level, Gingival recession, Mobility, Plaque index, Gingival index and Radiographic parameters (depth of intrabony defect) were recorded by a single investigator just before surgery as baseline data, and then were re-evaluated at 3 and 6 months postsurgery.

Probing pocket depth was measured as the distance from the gingival margin to the base of the pocket using UNC-15 probe. Relative attachment level and Gingival recession was measured with the same periodontal probe from a reference notch on an acrylic stent. Vertical grooves in the stent made proper alignment of the probe possible and ensured reliability and reproducibility for future comparisons.

To facilitate serial radiographic comparisons, intraoral periapical radiographs with attached X-ray grid, standardized by means of paralleling technique were utilized. The grid was calibrated in millimeters, which could be counted to measure the osseous defect fill on the radiograph.

PRE-SURGICAL PROTOCOL

After completing oral prophylaxis, subjects were re-evaluated after 4 weeks. The subjects showing acceptable oral hygiene were selected for the study and signed written consents were obtained from the patients.

SURGICAL PROTOCOL

Two of the bilateral defects were designated as control site (defect being treated with Biograft ® - HA ^{NANO}) and test site (defect being treated with Biograft ® - HA ^{NANO} and amnion membrane). Surgical procedure was performed under local anesthesia. Following buccal and

lingual intracrevicular incisions, full thickness mucoperiosteal flaps were raised. The defect was thoroughly debrided which was then prepared prior to grafting procedure.
Biograft ® - HA ^{NANO} was placed in the defect designated as control site and Biograft ® - HA ^{NANO} and amnion membrane was placed in defect designated as test site. The flaps were then replaced to their original position and sutured using direct loop 3-0 nonresorbable silk sutures. Coe Pak was placed over the treated site for protection, for a period of one week. Patients were instructed for the care of surgical site and to report back in case of any bleeding or any other adverse event.

ANTIBIOTICS
Amoxicillin 500 mg Lactobacilli 60 million spores, thrice daily for five days and Paracetamol 500 mg as and when required + Ibuprofen 400 mg thrice daily for three days and Chlorhexidine mouthrinse (0.12%) twice daily for 14 days. Subjects were recalled after 7 days for suture removal, patient motivation and oral hygiene instructions were reinforced at 1, 3 and 6 months recall visits. Clinical and radiographic parameters were re-evaluated at 3 and 6 months postsurgery.

STATISTICAL ANALYSIS
The statistical analysis was carried out using Statistical Package for Social Sciences (SPSS Inc., Chicago, IL, version 15.0 for Windows). All quantitative variables were estimated using measures of central location (mean) and measures of dispersion (standard deviation and standard error). For time related comparison Paired t-test was applied. All statistical tests were two- sided and performed at a significance level of α=.05.

RESULTS
Eighteen patients in age group 30-65 years (M:F of 10:8) had participated in this study. Eighteen intrabony defects were treated with hydroxyapatite and amnion membrane and eighteen with hydroxyapatite graft only. All the treated sites resulted in uneventful healing. No complications such as allergic reaction, abscesses, or infections were observed throughout the study period, in any of the patients.
Intergroup comparisons revealed that the difference in radiographic osseous defect fill was statistically significant for test group as compared to control group. When probing pocket depths of the control site were compared to the test site as shown in (**Table II),** statistically significant ($p<0.05$) mean difference of 1.22 mm and 0.72 mm was observed at 3 and 6 months respectively, which was in favor of the test site. Statistically significant mean CAL gains of 3.77±0.66 mm was observed in the test group and 2.78±1.09 mm in the control group from baseline to 6 months [Tables 2 and 3]. A decrease in score of papillary bleeding index by 0.77$\pm$0.64 mm was observed after 6 months postoperatively for the control group and 0.55$\pm$0.51mm after 6 months for the test group.
When mean increase in gingival recession for the control site was compared to the test site, a mean difference of 0.45 and 0.22 mm was observed at 3 and 6 months respectively, which were statistically non significant ($p>0.05$) **(Table IV**).
When the mean scores of dental plaque index of both the groups were compared with each other at different periods of observation, mean differences of 0.19, 0.19 and 0.13 were observed at baseline, 3 and 6 months postoperatively respectively, which were statistically non significant ($p>0.05$) **(Table VI)**.

Table I- Summary of the values of mean differences and mean percentage change in radiographic assessment between Control Site and Test Site at different periods of observation

Radiographic assessment (Mean %age change)			
RD_1-RD_2	28.17	27.90	0.27
RD_1-RD_3	50.76	53.87	-3.11
RD_2-RD_3	31.44	36.02	-4.58

*- Not Significant (p>0.05) * *- Significant (p<0.05)

RD_1: Radiographic osseous defect fill at baseline

RD_2: Radiographic osseous defect fill after 3 months

RD_3: Radiographic osseous defect fill after 6 months

1-2 (Change from baseline to 3months) *HS: Statistically highly significant.*

1-3 (Change from baseline to 6 months) *S: Statistically significant*

2-3 (Change from 3to 6months) *NS: Statistically non significant.*

Negative value indicates increase in mean score

Table II- Summary of mean differences and mean percentages of probing pocket depth between control and test site at different periods of observation

Time	CONTROL SITE Mean ± SD	TEST Site Mean ± SD	Mean difference	't' value	p value
Probing Pocket Depth (in mm)					
PPD_1	6.94 ± 2.48	7.44 ± 2.01	-0.5	0.6645	.5108*
PPD_2	5.0 ± 1.78	3.78 ± .65	1.22	2.7375	.0099**
PPD_3	3.83 ± 1.20	3.11 ± .32	0.72	2.4569	.0190**

Table III- Showing summary of the values of mean differences and mean percentages of relative attachment level between Control and Test site at different periods of observation

Time	CONTROL SITE Mean ± SD	TEST Site Mean ± SD	Mean difference	't' value	p value
Relative Attachment level (in mm)					
RAL_1	10.66 ± 2.63	10.83 ± 2.52	-0.17	.1860	.8535*
RAL_2	8.66 ± 2.30	6.78 ± 1.00	1.88	3.1972	.003**
RAL_3	6.88 ± 2.29	5.28 ± .96	1.6	2.7407	.0097**

Table IV- Summary of the values of mean differences and mean percentages of gingival recession between Control and Test site at different periods of observation

Time	CONTROL SITE Mean ± SD	TEST Site Mean ± SD	Mean difference	't' value	p value
Gingival Recession (in mm)					
GR_1	6.05 ± 2.41	5.22 ± 1.55	0.83	1.2289	.2275*
GR_2	5.5 ± 1.65	5.05 ± 1.83	0.45	.581	.4438*
GR_3	4.77 ± 1.26	4.55 ± 2.00	0.22	.3949	.6954*

Table V: Showing summary of the values of mean differences in papillary bleeding index between Control and Test Site at different periods of observation

Time	CONTROL SITE Mean ± SD	TEST Site Mean ± SD	Mean difference	't' value	p value
Papillary Bleeding Index (in mm)					
BI_1	1.33 ± 0.46	2.27 ± 0.63	0.94	5.1125	<.0001***

BI_2	1.66 ± 0.76	1.16 ± 0.51	0.50	2.3177	.0266**
BI_3	0.77 ± 0.64	0.55 ± 0.51	0.22	1.1406	.2620*

Table VI: Showing summary of the values of mean differences in Dental Plaque Index between Control and Test Site at different periods of observation

Time	CONTROL SITE Mean ± SD	TEST Site Mean ± SD	Mean difference	't' value	p value
Dental Plaque Index					
PI_1	2.44 ± 0.70	2.25 ± 0.49	0.19	1.014	.686*
PI_2	1.86 ± 0.54	1.67 ± 0.59	0.19	1.020	.687*
PI_3	1.69 ± 0.79	1.56 ± 0.86	0.13	1.004	.652*

Fig 1: Radiographic osseous defect fill at baseline

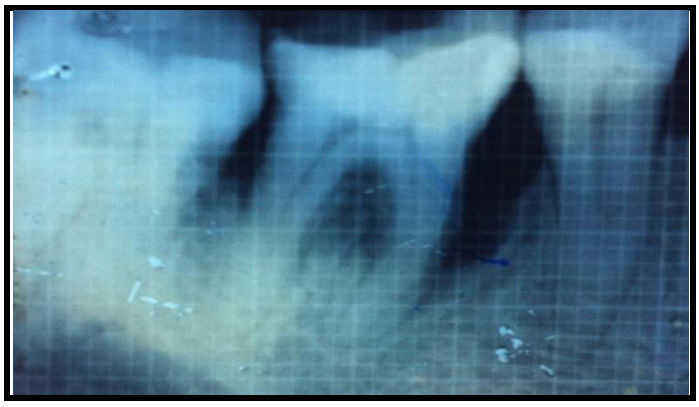
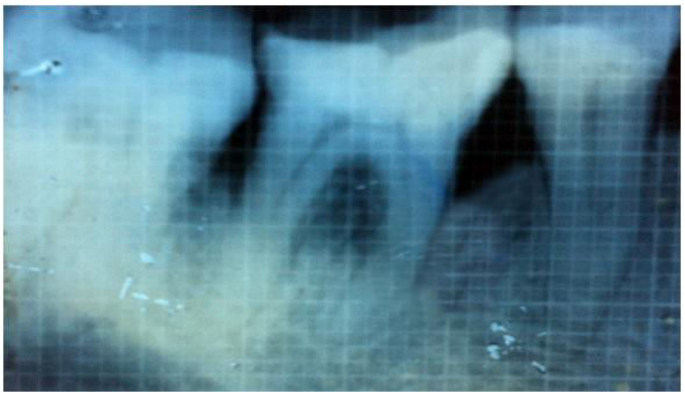


Fig 2: Radiographic osseous defect fill after 6 months



DISCUSSION

Successful periodontal regeneration relies on the reformation of an epithelial seal, deposition of new acellular extrinsic fiber cementum and insertion of functionally oriented connective tissue fibers in to the root surface and restoration of alveolar bone height[7].

Among the natural resorbable membranes, Amnion membrane is new to dentistry. Amnion is a processed, dehydrated and sterilized graft of human placenta. This membrane differs from other membranes currently available in the fact that it has inherent anti-inflammatory and anti-bacterial properties. Additionally, this membrane also contains a high concentration of laminin-5 through out the amnion membrane **Xenoudi P, Lucas M (2011).**

Laminin-5 is a protein with a high affinity for cellular adhesion of gingival epithelial cells, providing a bioactive matrix for cellular migration. This allows for rapid sealing of the underlying graft material used for the treatment of intrabony defects. Furthermore,

immunohistochemical stain analysis has also shown that this membrane contains growth factors such as platelet derived growth factors alpha and beta (PDGF-α, PDGF-α) as well as transforming growth factor beta (TGF-β) **Xenoudi P, Lucas M (2011).**

In **control group,** a mean probing pocket depth reduction of 3.11mm was observed from baseline to 6 months, which was statistically significant ($p<0.001$)**.** The results of this study are in agreement with the previous study by **Mohammad Taghi Chitsazi et al 2011** who had also reported significant reduction in probing pocket depth in the sites treated with nano-crystalline hydroxyapatite bone graft.[8] In **test group**, mean probing pocket depth reduction of 4.33 mm was observed from baseline to 6 months, which was statistically significant ($p<0.001$)**.** Similarly studies done by **Paul S. Rosen (2009) and Dan Holtzclaw et al (2009)** using combination of bone graft and amnion membrane also showed similar results which were comparable to the results of the present study.[9] When the reduction in mean probing pocket depth was compared for both the groups, the **test group** showed more reduction in mean probing pocket depths than the **control group** from baseline to 6 months which were statistically significant ($p<0.05$).

Relative attachment level gain was statistically significant from baseline to 6 months for both the groups. This gain in attachment level can be attributed to periodontal regeneration, long junctional epithelium formation and/or soft tissue healing at the base of the pocket. In **control group,** a mean attachment level gain of 3.78mm was observed from baseline to 6 months, which was statistically significant ($p<0.001$)**.** The results of this study are in agreement with the previous study by **Mohammad Taghi Chitsazi et al 2011** who had also reported significant gain in attachment level in the sites treated with nano-crystalline hydroxyapatite bone graft[8]. In **test group**, mean attachment level gain of 5.55 mm was observed from baseline to 6 months, which was statistically significant ($p<0.001$)**.** Similarly studies done by **Paul S. Rosen (2009) and Dan Holtzclaw et al (2009)** using combination of bone graft and amnion membrane also showed similar results which were comparable to the results of the present study[9].

When mean attachment level gain was compared for both the groups, the **test group** showed more mean attachment level gain than the **control group** from baseline to 6 months which were statistically significant ($p<0.05$).

Gingival recession increased substantially over a period of 6 months after surgery. In **control group,** an increase in gingival recession of 1.28 mm was observed from baseline to 6 months, which was statistically significant ($p<0.001$)**.** These changes may be attributed to the shrinkage of gingival tissues with the resolution of inflammation. The results of this study are in agreement with the previous study by **Mohammad Taghi Chitsazi et al 2011** who had also reported significant increase in gingival recession in the sites treated with nano-crystalline hydroxyapatite bone graft[8]. In **test group**, mean increase in gingival recession of 0.67 mm was observed from baseline to 6 months, which was statistically significant ($p<0.001$)**.** Similarly studies done by **Paul S. Rosen (2009) and Dan Holtzclaw et al (2009)** using combination of bone graft and amnion membrane also showed similar results which were comparable to the results of the present study.[9] When increase in gingival recession was compared for both the groups, the **control group** showed more mean increase in gingival recession than the **test group** from baseline to 6 months which were statistically non significant ($p<0.05$).

Plaque index was monitored throughout the study period. Values are almost same for both the groups. This variable is totally dependent on the patient's compliance and his/her efficacy to maintain oral hygiene. As the subjects were on continuous periodic recall, constant motivation, education and oral hygiene instructions revision have led to almost similar plaque scores at all the periods of observation, which have negated the possibility of the elucidation of effect of this variable on regeneration. No significant changes in plaque scores have also been reported in various previous studies by **Subbaiah and Thomas 2011, Zamet, Darbar and Griffiths 1997.**

Mean reduction in papillary bleeding index scores were observed for both the groups after 3 months. This may be attributed to the resolution of inflammation and return of the gingival tissues from a diseased state to health. After 6 months there was significant decrease in scores for both the groups. In **control group,** a mean reduction in papillary bleeding index of 0.56mm was observed from baseline to 6 months, which was statistically significant ($p<0.001$)**.** The results of this study are in agreement with the previous study by **Mohammad Taghi Chitsazi et al 2011** who had also reported significant reduction in papillary bleeding index in the sites treated with nano-crystalline hydroxyapatite bone graft.[8] In the **test group,** mean reduction in papillary bleeding index of 1.72 mm was observed from baseline to 6 months, which was statistically significant ($p<0.001$)**.** Similarly studies done by **Paul S. Rosen (2009) and Dan Holtzclaw et al (2009)** using combination of bone graft and amnion membrane also showed similar results which were comparable to the results of the present study.[9] When the reduction in mean papillary bleeding index was compared for both the groups, the **test group** showed more reduction in mean papillary bleeding index than the **control group** from baseline to 6 months which were statistically significant ($p<0.05$).

In **control group,** the amount of mean radiographic osseous defect bone fill of 50.76% (2mm) was observed from baseline to 6 months, which was statistically significant ($p<0.001$)**.** The results of this study are in agreement with the previous study by **Mohammad Taghi Chitsazi et al 2011** who had also reported significant increase in radiographic osseous defect fill in the sites treated with nano-crystalline hydroxyapatite bone graft.[8] In **test group,** mean bone fill of 53.87% (2.78mm) was observed from baseline to 6 months, which was statistically significant ($p<0.001$)**.** Similarly studies done by **Paul S. Rosen (2009) and Dan Holtzclaw et al (2009)** using combination of bone graft and amnion membrane also showed similar results which were comparable to the results of the present study.[9] When mean bone fill was compared for both the groups, the **control group** showed -3.11% (0.53mm) greater bone fill as compared to test group after 6 months, which was statistically non significant ($p>0.05$).

CONCLUSION

Amnion membrane resulted in statistically significant improvements in radiographic osseous defect measurements and clinical parameters. It was very well tolerated by the subjects. No adverse effects such as periodontal abscess, inflammation and/or allergic reaction in the treated surgical sites were reported. Although the clinical parameters i.e probing pocket depth reduction, clinical attachment level gain and radiographic evidence of bone fill are proved to be consistent with the successful regenerative therapy, but these findings cannot be directly extrapolated as an outcome of periodontal regeneration, as these are not supported by histologic evidence.

So future studies with more critically designed protocols, larger sample size and inclusion of histologic evidence as a criteria for periodontal regeneration, are warranted to further explore the potential of the Amnion membrane as a periodontal regenerative material.

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