Comparative Evaluation Of Amniotic Membrane Versus Chorionic Membrane In Combination With Nano-Crystalline Hydroxyapatite Bone Graft In Management Of Infrabony Defect: A Clinico-Radiographic Study

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**ABSTRACT**

**Introduction:** Recently the use of biologic membranes and growth factors has significantly increased in Regenerative therapy. One of the oldest biomaterials used for periodontal regeneration are Placental membranes which consist of Amniotic and Chorionic membrane. Hence, the purpose of the present study is to compare and evaluate the efficacy of the Amniotic membrane and Nano-crystalline hydroxyapatite bone graft versus Chorionic membrane with Nano-crystalline hydroxyapatite bone graft in the treatment of infrabony defects.

**Materials and Method:** A total of 16 sites with infrabony defects were assigned to two groups (Group A – Amniotic membrane and Nano-crystalline hydroxyapatite bone graft and Group B – Chorionic membrane and Nano-crystalline hydroxyapatite bone graft). Parameters such as Probing Pocket Depth (PPD), Clinical attachment level (CAL), Measurement of Intraosseous Component (IC), Plaque Index (PI), Modified Gingival Index (MGI) were measured at baseline and after 3 and 6 months. The significance of difference within and between the groups was evaluated with paired and unpaired t-tests. **Results:** The mean PI, MGI, PPD, CAL and IC showed a statistically significant difference from baseline to 3 and 6 months (p≤0.01) interval in Group A as well as in Group B. Statistically no significant difference was found between both the groups at baseline, three months and six months. **Conclusion:** Both the Amniotic and Chorionic membrane seem to be favorable allograft to be used in Infrabony defects.

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INTRODUCTION:
The destruction of alveolar bone around the teeth is an important sign of advanced periodontal disease. Moreover, it is an indicator of apical spread of the disease. Bony defect occurring in oblique direction alongside the root surface and having a base located in the apical of the alveolar crest is called ‘infrabony’ or ‘intraosseous’ defect.¹
The successful management of sites with infrabony defects is an extremely challenging procedure as failure of which may cause greater amount of attachment loss. In 1976 Melcher² suggested that type of new attachment on the root surface is determined by the cells which can repopulate the periodontal wounds. Based on his theory, it was reported that the placement of physical barriers in between root and epithelium will retard the apical migration of epithelial cells; thereby facilitating healing by the cells of periodontal ligament and adjacent alveolar bone this procedure was named as Guided Tissue Regeneration (GTR).

Cellulose acetate used by Nyman et al in 1982 was the first membrane.³ Since then, wide range of new membranes including absorbable and nonabsorbable membranes have been developed. Recently, biologic membranes and growth factors have been used extensively to improve healing. One of the oldest biomaterials used for scaffolds is the placental membrane. The placental membranes are comprised of two types of membranes namely amniotic and chorionic tissues.

Amniotic membrane (AM) is a biologic membrane, which is capable of periodontal regeneration. It is innermost placental layer, composed of single epithelial layer, avascular stroma and thick basement membrane. This membrane possess anti-inflammatory and antimicrobial effects and is a good source of stem cells thus improving healing through reduction of post operative scarring. It plays a important role in improved healing of periodontal lesions and might result in reduction of Probing Pocket Depth (PPD) and Clinical Attachment loss.⁴⁵

Chorion is the outermost layer of the placental membrane which consists of a spongy layer.⁶ It is a well-tolerated, biodegradable membrane. It is widely used since they are provided with property of being immune privileged.⁷ Chorionic membrane (CM) acts as a barrier membrane between the root surface and the gingival epithelium, which promotes the periodontal ligament cells to form progenitor cells that can regenerate new tissues.

In an attempt to further improve the clinical outcome of GTR techniques, particularly in unfavorable and large defects, the use of various grafting materials underneath the membrane has been suggested; this association is currently called Combined Periodontal Regenerative Technique (CPRT).⁸ Bone graft materials support the membrane by helping it to retain the desired volume and to promote osteoconductive properties. In the present study Nano-crystalline hydroxyapatite bone graft (NcHA) was used along with placental membranes. Advantages of NcHA material are osteoconductivity, bioresorbablity, and close contact. NcHA bind to bone and stimulate bone healing by stimulation of osteoblast activity.⁹

This study was designed to evaluate and compare efficacy of the Amniotic and Chorionic membrane with Nano-crystalline hydroxyapatite bone graft in treatment of Infrabony defects.

MATERIALS AND METHOD:
Eight patients, 4 males and 4 females (20–60 years old; mean age 43.12 years) with Infrabony defects were selected in the study. It was a split-mouth design study. The ethical clearance was obtained from the institutional ethical committee. The informed consent was taken from patients after explaining the procedure in their own language. No patients had any contraindications to periodontal surgery. The inclusion criteria were: 1) Patients with the age range of 20-60 years 2) Patients with good systemic health 3) Probing pocket depth more than 5 mm bilaterally 4) Radiographic evidence of vertical defect bilaterally.

Clinical & Radiographical data:
The following clinical and radiographic measurements were recorded at baseline, 3 months and 6 months postsurgery for all the sites:

1. Turesky- Gilmore- Glickman modification of the Quingly- Hein plaque index (PI) Turesky et al, 1970¹⁰
2. Modified gingival index (MGI) Lobene et al, 1986¹¹
3. Probing Pocket Depth (PPD)
4. Clinical Attachment Level (CAL)
5. Intraosseous component (IC) was obtained with the help of Radiovisiography (RVG) with
grid by measuring the distance from CEJ to the base of the defect (CEJ – BD) - distance from CEJ to the alveolar crest (CEJ-BC). The amount of bone fill was obtained by intraosseous component at six months – Intraosseous component at baseline.

Patients who participated in the study initial therapy were performed, consisting of scaling and root planning followed by oral hygiene instructions. After 4–6 weeks following initial therapy, patient were reevaluated for Periodontal Pocket, CAL and oral hygiene maintenance following which periodontal pocket therapy was performed. Quadrants were divided into two study sites (Group A and Group B) in a split-mouth design. The Group A was treated by Open flap debridement (OFD) with Amniotic membrane and Nanocrystalline hydroxyapatite bone graft (figure 1) and the Group B was treated by OFD with Chorionic membrane and Nanocrystalline hydroxyapatite bone graft (figure 2). All the surgeries were performed by the same operator. The AM and CM was obtained from, Tata Memorial Hospital Tissue Bank, Mumbai, India.

**Surgical Procedure:**

Following all the preclinical measurements, intraoral antisepsis was performed with 0.2% chlorhexidine digluconate rinse. After administrating local anesthesia (2% lignocaine in the ratio of 1:80,000) and achieving proper anesthesia, intrasulcular and interdental incisions were given using 15 size BP blade. Full thickness mucoperiosteal flap were elevated and root debridement and curettage was done using area specific Gracey curettes. Intrapony defect was identified. The surgical area was irrigated with sterile normal saline. Pre-suturing was done. In the group A, Amniotic membrane was placed after packing the infrabony defect with Nanocrystalline hydroxyapatite bone graft. Flap was sutured and surgical area was protected and covered with Coe-pak. In the group B, Chorionic membrane was placed after packing the infrabony defect with nanocrystalline hydroxyapatite bone graft. Flap was sutured and surgical area was protected and covered with Coe-pak. Patients received post-operative instructions and were prescribed antibiotic and analgesics. Patients were instructed to rinse twice daily with 0.2 % chlorhexidine gluconate for 2 weeks. The periodontal dressing and sutures were removed 10 days postsurgery. The patients were recalled at 3 and 6 months postoperatively to evaluate oral hygiene status. At 6 months all the parameters were reassessed.

**Figures:**

(1) Pre treatment photographs, (2) Measurement Probing Pocket Depth, (3) Radiographic measurement of Intraosseous component, (4) Flap Reflected exposing defect site and debridement done, (5) Bone grafting done, (6) Amniotic membrane placed, (7) Sutures taken, (8-9) Follow-up after 3 months, (10-11) Follow-up after 6 months.
Statistical analysis

Data were tabulated and analyzed using SPSS Version 23 software. For the intergroup comparison, an unpaired t-test was used, whereas a paired t-test was applied for intragroup comparison. The mean and standard deviation values of all the parameters were calculated at baseline, 3 and 6 months postoperatively. Differences were considered statistically significant at \( P < 0.05 \) and \( P \leq 0.001 \) was considered very highly significant.

**RESULTS:**

In this randomized controlled clinical trial, both the groups showed improvement in all the clinical recorded parameters.

The mean value results from paired t test (Intra group) showed statistically significant difference in PI, MGI, PPD, CAL and IC in both the groups at baseline to three months and six months postoperatively.

**Table 1: Intragroup comparison of difference in clinical and radiographic parameters from baseline to 6 months in Group A (OFD with Amniotic membrane and Nanocrystalline hydroxyapatite bone graft) and Group B (OFD with Chorionic membrane and Nanocrystalline hydroxyapatite bone graft)**

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Group</th>
<th>Mean difference</th>
<th>( P )</th>
</tr>
</thead>
<tbody>
<tr>
<td>PI</td>
<td>Group A</td>
<td>1.06</td>
<td>( \leq 0.001)****</td>
</tr>
<tr>
<td></td>
<td>Group B</td>
<td>1.06</td>
<td>( \leq 0.001)****</td>
</tr>
<tr>
<td>MGI</td>
<td>Group A</td>
<td>1.73</td>
<td>( \leq 0.001)****</td>
</tr>
<tr>
<td></td>
<td>Group B</td>
<td>1.72</td>
<td>( \leq 0.001)****</td>
</tr>
<tr>
<td>PPD</td>
<td>Group A</td>
<td>3.50</td>
<td>( \leq 0.001)****</td>
</tr>
<tr>
<td></td>
<td>Group B</td>
<td>4.12</td>
<td>( \leq 0.001)****</td>
</tr>
<tr>
<td>CAL</td>
<td>Group A</td>
<td>3.62</td>
<td>( \leq 0.001)****</td>
</tr>
<tr>
<td></td>
<td>Group B</td>
<td>4.25</td>
<td>( \leq 0.001)***</td>
</tr>
<tr>
<td>IC</td>
<td>Group A</td>
<td>2.63</td>
<td>( \leq 0.001)****</td>
</tr>
<tr>
<td></td>
<td>Group B</td>
<td>2.88</td>
<td>( &lt; 0.001)****</td>
</tr>
</tbody>
</table>
Test applied: Paired t-test, Very Highly significant (p≤0.001)****, Highly significant (p≤0.01)***, Significant (p≤0.05)**, Nonsignificant (p>0.05)*, PI – Plaque index; MGI – Modified Gingival index; PPD- Probing Pocket Depth, CAL – Clinical Attachment Level, IC – Intraosseous Component, SD – Standard deviation; P – Probability

The results from Un-paired t test (Inter group) showed statistically significant difference in mean PI, MGI, PPD, CAL and IC between both the groups at baseline to three months and six months postoperatively.

### Table 2: Intergroup comparison of clinical and radiographic parameters at baseline, 3 months and 6 months between Group A (OFD with Amniotic membrane and Nanocrystalline hydroxyapatite bone graft) and Group B (OFD with Chorionic membrane and Nanocrystalline hydroxyapatite bone graft)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Duration</th>
<th>Group A</th>
<th>Group B</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>SD</td>
<td>Mean</td>
<td>SD</td>
</tr>
<tr>
<td>PI</td>
<td>Baseline</td>
<td>2.00</td>
<td>0.30</td>
<td>2.00</td>
</tr>
<tr>
<td></td>
<td>3 months</td>
<td>1.13</td>
<td>0.07</td>
<td>1.14</td>
</tr>
<tr>
<td></td>
<td>6 months</td>
<td>0.94</td>
<td>0.06</td>
<td>0.94</td>
</tr>
<tr>
<td>MGI</td>
<td>Baseline</td>
<td>2.31</td>
<td>0.58</td>
<td>2.31</td>
</tr>
<tr>
<td></td>
<td>3 months</td>
<td>0.68</td>
<td>0.05</td>
<td>0.68</td>
</tr>
<tr>
<td></td>
<td>6 months</td>
<td>0.58</td>
<td>0.07</td>
<td>0.59</td>
</tr>
<tr>
<td>PPD</td>
<td>Baseline</td>
<td>6.63</td>
<td>0.91</td>
<td>7.00</td>
</tr>
<tr>
<td></td>
<td>3 months</td>
<td>4.00</td>
<td>0.75</td>
<td>4.13</td>
</tr>
<tr>
<td></td>
<td>6 months</td>
<td>3.13</td>
<td>0.64</td>
<td>2.88</td>
</tr>
<tr>
<td>CAL</td>
<td>Baseline</td>
<td>7.00</td>
<td>0.92</td>
<td>7.38</td>
</tr>
<tr>
<td></td>
<td>3 months</td>
<td>4.25</td>
<td>0.70</td>
<td>4.50</td>
</tr>
<tr>
<td></td>
<td>6 months</td>
<td>3.38</td>
<td>0.91</td>
<td>3.13</td>
</tr>
<tr>
<td>IC</td>
<td>Baseline</td>
<td>3.88</td>
<td>0.64</td>
<td>3.88</td>
</tr>
<tr>
<td></td>
<td>3 months</td>
<td>2.37</td>
<td>0.51</td>
<td>2.56</td>
</tr>
<tr>
<td></td>
<td>6 months</td>
<td>1.25</td>
<td>0.46</td>
<td>1.00</td>
</tr>
</tbody>
</table>

Test applied: Unpaired t-test; Values expressed as means±SD. *P>0.05 was considered as not significant; *P<0.05 was considered as significant. PI – Plaque index; MGI – Modified Gingival index; PPD- Probing Pocket Depth, CAL – Clinical Attachment Level, IC – Intraosseous Component, SD – Standard deviation; P – Probability

**Graph 1:** Mean changes in Probing Pocket Depth (PPD) between Group A and Group B at baseline, 3 months and 6 months
DISCUSSION:
One of the goals in periodontal therapy is to restore periodontal tissues lost through periodontal disease. The regeneration of the periodontium is the result of elective cellular events that are facilitated by tissue exclusion using bioabsorbable or non-resorbable barriers. The results of our study showed that both Amniotic and Chorionic membranes were equally effective in treatment of infrabony defects.

In the present study, full mouth PI and MGI scores remained low throughout the study period. This reduction in scores could be attributed to the regular oral hygiene instructions given to the patients thereby enabling improved plaque control efficiency and better patient compliance, generally observed following periodontal surgery. Similar results were obtained in the study done by Kothiwale SV in 2014\textsuperscript{12}, Kumar et al in 2015\textsuperscript{13} and Kothiwale S in 2018\textsuperscript{14} this could be due to oral prophylaxis and periodontal pocket therapy. In the present study, the mean PPD for Group A at baseline was 6.63±0.91 mm which reduced to 4.00±0.75 mm and 3.13±0.64 mm at 3 months and 6 months postoperatively. These results were similar to those reported by Kothiwale et al 2009\textsuperscript{7}, Kiany et al 2015\textsuperscript{5}, Kumar et al 2015\textsuperscript{13} and Sali DD in 2016\textsuperscript{15}. In the present study, the mean PPD for Group B at baseline was 7.00±1.30 mm which reduced to 4.13±0.83 mm and 2.88±0.64 mm at 3 months and 6 months postoperatively. Similar results were observed by Kothiwale SV 2014\textsuperscript{12}, Kothiwale S 2018\textsuperscript{14} and Tarini K 2018\textsuperscript{16}. In the present study, statistically no significant difference in mean PPD was seen between both the groups at baseline (p>0.05), 3 months ((p>0.05) and 6 months (p>0.05).
In the present study, the mean CAL for Group A at baseline was 7.00±0.92 mm which reduced to 4.25±0.70 mm and 3.38±0.91 mm at 3 months and 6 months postoperatively. Similar results were observed by Kothiwale et al 2009, Kiany et al 2015, Kumar et al 2015 and Sali DD 2016. In the present study, the mean CAL for Group B at baseline was 7.38±1.18 mm which reduced to 4.50±0.75 mm and 3.13±0.99 mm at 3 months and 6 months postoperatively. Similar results were observed by Kothiwale SV 2014, Kothiwale S 2018 and Tarini K 2018. In the present study, statistically no significant difference in mean CAL was seen between both the groups at baseline (p>0.05), 3 months (p>0.05) and 6 months (p>0.05).

In the present study, the mean Intraosseous Component (IC) for Group A at baseline was 3.88±0.64 mm which reduced to 2.37±0.51 mm and 1.25±0.46 mm at 3months and 6months postoperatively. In the present study amount of bone fill for group A was 2.63 mm. Similar results were observed by Kothiwale et al 2009 (2.1 mm ± 0.36), Agarwal et al 2015 (3.28 mm) and Sali DD 2016 (1.78 ± 0.04 mm). In the present study, the mean IC for Group B at baseline was 3.88±0.83 mm which reduced to 2.56±0.49 mm and 1.00±0.75 mm at 3months and 6months postoperatively. In the present study amount of bone fill for group A was 2.88 mm. Kothiwale SV 2014 did a study using Chorionic membrane with Open flap debridement procedure in which the amount of bone fill found was 1.22 ± 0.35 mm. In the present study, statistically no significant difference in mean IC was seen between both the groups at baseline (p>0.05), 3 months (p>0.05) and 6 months (p>0.05).

The results of this study showed that both Amniotic and Chorionic membrane seem to be favorable allografts to be used in Infrabony defects. Both the membranes have good self life, easy availability, support in healing and cost effective as compared to other membranes. Due to its greater thickness and density; Chorionic membrane was easy to handle as compared to Amniotic membrane clinically. There are a few limitations of this study in terms of negative impact on the sample size of study; it also delayed in data collection and follow-up period due to COVID pandemic. But still more numbers of studies with larger sample size and for longer duration are needed to be conducted to evaluate the potential of Amniotic membrane and Chorionic membrane in terms of clinical advantages as well as histological analysis of healing.

CONCLUSION:
Both the materials, Amniotic membrane and Chorionic membrane in combination with Nano-crystalline hydroxyapatite bone graft can be considered as effective materials in the treatment of Infrabony defects. Due to its greater thickness and density; Chorionic membrane was easy to handle as compared to Amniotic membrane clinically. Both Amniotic membrane and Chorionic membrane seem to be promising novel tissue engineered biomaterials. Rich source of stem cells, enhancement of healing and self-adhering property make these membranes an effective option for Periodontal Regeneration.

Further research and long term clinical trials investigating the full potential of Amniotic membrane and Chorionic membrane in infrabony defects are still warranted to strengthen the fact that Amniotic membrane and Chorionic membrane are indeed superior choices for the treatment of Infrabony defects.

REFERENCES

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