SOFT TISSUE HEALING AROUND IMMEDIATELY PLACED DENTAL IMPLANTS AUGMENTED WITH AMNIOTIC CHORION MEMBRANE VERSUS CONNECTIVE TISSUE GRAFT
(A CLINICAL AND HISTOLOGICAL STUDY)
Norai A. Zayed1 BDS, Ahmed M. Hommos2 PhD, Sahar SH. Karam3 PhD, Rania A. Fahmy4 PhD.

ABSTRACT

INTRODUCTION: The Amnion Chorion Membrane (ACM) has favorable regenerative properties which facilitates its use for covering immediate dental implants.

OBJECTIVES: To evaluate clinically and histologically the effect of Amnion Chorion membrane in augmenting immediately placed dental implants compared to connective tissue graft.(CTG)

MATERIALS AND METHODS: This study was conducted clinically on eighteen upper hopeless anterior/premolar teeth scheduled for extraction and immediate implant placement. Patients were divided into two groups: in the first group, nine immediate implants were covered with ACM, and in the second group nine immediate implants were covered with CTG. Keratinized mucosal width (KMW), keratinized mucosal thickness (KMT) and healing score were recorded for both groups at 7, 14, 30 and 90 days.

RESULTS: The KMT showed an increase by 26% in the test group while in the control group it decreased by 6.5% by the end of the study period. Regarding the healing scores both groups showed marked improvement throughout the study, lower values of healing scores were noted in the test group. Clinical results were confirmed by histological examination which revealed that oral mucosa of both groups showed parakeratinized gingival epithelium. The connective tissue of the test group showed more regularly arranged, thick bundles.

CONCLUSIONS: Both treatment modalities were satisfactory but the ACM offered more positive results concerning the mucosal thickness and healing scores.

KEYWORDS: Immediate implants, ACM, Connective tissue graft, Soft tissue.
fibronectin and laminin (6). Collagen is well tolerated and bioabsorbable, has hemostatic properties, and encourages migration of adjacent autogenous connective tissue. Fibronectin is involved in many cellular processes, including tissue repair, blood clotting, cell migration, and adhesion (7). Laminin and Laminin-5 has a high affinity for binding epithelial cells, and in contrast to traditionally available membranes (8). This biological factor allows the ACM to be left exposed to the oral environment. It has been also proven that the ACM has a potential for regeneration in periodontology as the matrix of the chorion contains abundant growth factors, such as keratinocyte growth factor, basic fibroblast growth factor, and transforming growth factor-β, that promote periodontal regeneration and provide a natural environment for accelerated healing (9). It was used in socket preservation, guided tissue regeneration and guided bone regeneration. Furthermore, the ability of this allograft to self-adhere eliminates the need for suturing (10).

The present study was performed to compare and evaluate clinically and histologically the soft tissue healing around immediately placed dental implants augmented with Amnion Chorion Membrane versus sub epithelial connective tissue graf.

MATERIALS AND METHODS

The present study was conducted on eighteen upper anterior/premolar hopeless teeth that have been scheduled for extraction attending the outpatient clinic of the Periodontology and Oral Medicine Department, Faculty of Dentistry, Alexandria University.

The study was approved by the ethical committee at the Faculty of Dentistry, Alexandria University. All patients received both oral and written information about the study protocol and signed their informed consent before agreeing to participate in the study.

Inclusion criteria:
The patients were systemically healthy (American Society of Anesthesiologists I or II) (11) with age ranging between (20-40) years.

All patients had a tooth in the maxillary premolar or anterior region requiring extraction due to one of the following reasons:
1- Badly decayed tooth that cannot be restored.
2- Tooth with failed endodontic treatment.
3- Tooth with longitudinal fracture.

Exclusion criteria
Unstable systemic diseases precluding surgical procedures, compromised healing conditions (e.g., uncontrolled diabetes mellitus or human immunodeficiency virus infection), bone disorders (hyperparathyroidism, osteoporosis, or Paget’s disease), smoking more than 10 cigarettes per day, long-term (>2 weeks) use of medications that affect the periodontium eg: (anti-inflammatories, steroids, or bisphosphonates) in the past 3 months. Pregnant or lactating women. Besides, an O’Leary plaque score > 20% (12) and localised or generalised aggressive periodontitis

Pre surgical phase
1. The assessment of oral hygiene condition of the patient, the condition of the tooth or remaining root to be extracted, the recipient site and the inter-occlusal space.
2. Radiographic evaluation: (Cone beam computed tomography (C.B.C.T) ) was performed at the first visit to evaluate:
   - Location of implant recipient site away from any vital anatomical structures.
   - Presence of adequate bone quantity and quality
3- Impression for the upper jaw of the patient using alginate impression material (Hydrogum, Zhermack, Italy) to fabricate transparent acrylic stent for measurement of the keratinized -mucosal thickness.
4. Oral hygiene instructions were given to the patients which include teeth brushing using a proper technique 3 times daily.

Surgical Phase

Tooth extraction
- The tooth was atraumatically extracted using a periotome (Nordent, USA) which acts by severing the periodontal ligaments avoiding bucco-palatal movements to avoid damage to the buccal plate of bone.

Implant Placement
- Drilling with low speed (1000 rpm), high torque and internal irrigation with normal saline to maintain bone vitality was performed to depth of 3 mm beyond the socket.
- Implant ( Dio implant, Korea ) was threaded into the bone using a ratchet as recommended by the manufacturer.
- The implant was seated subcrestally 2 mm below the crest of the socket wall.
- Adequate primary stability was obtained.

For test group

ACM ( Bioxclude , Snoasis Medical, USA ) was applied to augment the immediate implant site extending 2-3 mm under the buccal and palatal flaps leaving 2 mm of it exposed (Figure 1).

Figure 1: ACM membrane placed over the implant and tucked beneath the partial thickness buccal and palatal flaps.

Flap was sutured back into position to provide proper adaptation of the ACM by crossed over -horizontal mattress sutures using 4-0 non resorbable sutures leaving 2 mm of
the ACM exposed. There was no coronal advancement of the labial flap to maintain the integrity of the mucogingival line for good esthetics.

For control group

Connective tissue graft of approximately 1.5-2 mm thickness was harvested from the palate of the same site of implant placement, following local anesthesia. A "trap door" split thickness flap, consisting of 1 horizontal and 2 vertical incisions, was elevated (13) (Figure 2).

**Figure 2:** Harvesting CT graft using trap door technique.

The underlying connective tissue was harvested using a periosteal elevator.

Connective tissue graft was secured over the implant (Figure 3)

**Figure 3:** CT graft covering implant replacing upper left central incisor.

using 4-0 non resorbable horizontal mattress sutures. Closure of the flap by interrupted sutures was performed if needed.

Follow up phase

I) Clinical evaluation

Follow up was performed at base line, seven days, fourteen days, one month, and three months respectively for evaluating:

1. Keratinized mucosal width measured using William’s periodontal probe at baseline, one and three months.

2. Keratinized mucosal thickness measured, at baseline, one month and three months, using a stent where holes were located at the midpoint on the mid-facial surface of the mucosa. A periodontal probe was be inserted through the hole perforating the mucosa all the way to the cortical bone.

3. Healing score was taken according to (AL-Mashhadani, 2012) (16):

   4 = Necrotic Tissue (Eschar): black, brown, or tan tissue that adheres firmly to the wound bed

   3 = Slough: yellow or white tissue that adheres to the wound bed

   2 = Granulation Tissue: pink or beefy red tissue with a shiny, moist, granular appearance.

   1 = Epithelial Tissue: for superficial ulcers, new pink or shiny tissue that grows in from the edges or as islands on the wound surface.

   0 = Closed/Resurfaced: the wound is completely covered with epithelium.

II) Histological evaluation

A tissue sample 3 mm in size was obtained by a tissue punch during the second stage surgery to uncover the implant for abutment insertion (4 months). All specimens were labeled and fixed in 10% neutral buffered formalin. After fixation, specimens were dehydrated in ascending concentrations of ethanol, cleared with xylene, infiltrated and embedded in paraffin wax. Thin sections of 5 µm thick were cut using rotary microtome. Sections were stained with Haematoxylin and Eosin stains (H & E) for general examination.

Statistical analysis (15)

Data were fed to the computer and analyzed using IBM SPSS software package version 20.0. (Armonk, NY: IBM Corp) (16). Qualitative data were described using number and percent. The Kolmogorov-Smirnov test was used to verify the normality of distribution Quantitative data were described using range (minimum and maximum), mean, standard deviation and median. Significance of the obtained results was judged at the 5% level

RESULTS

Clinical evaluation (Figure 4)

A significant regression in the KMW in the study group was realized after one month compared to baseline (from a mean of 5.73±0.71 to a mean of 4.90±1.14) (p=0.34) followed by an insignificant increase to a mean of 4.96±1.21 after three months. However, in the control group a decrease in the KMW was evident at one month with a mean (4.77±1.36) and 3 months with a mean of (4.73±1.40). This decrease was insignificant both at baseline to 1 month and from 1 month to 3 months. However, it was significant from baseline to 3 months (p=0.049). On comparing both groups together no significant difference was realized throughout different studied periods (Table 1).
Moreover, in the control group there was a decrease in the keratinized mucosal thickness from baseline to one month (from a mean of 3.44±1.01 to mean of 3.22±1.09) which continued to decrease up till three months (from a mean of 3.22±1.09 to a mean of 3.11±0.93). There was no statistical significance between all studied periods (Table 2).

Table (2): Descriptive analysis of the studied cases with respect to keratinized mucosal thickness (in mm.) in two groups.

<table>
<thead>
<tr>
<th>Keratinized mucosal thickness (mm.)</th>
<th>Baseline</th>
<th>1 month</th>
<th>3 months</th>
<th>p_t</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Test group (n=9)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean ± SD.</td>
<td>3.56 ± 1.59</td>
<td>3.89 ± 0.78</td>
<td>3.67 ± 0.50</td>
<td>0.540</td>
</tr>
<tr>
<td>Min. – Max.</td>
<td>1.0 – 6.0</td>
<td>3.0 – 5.0</td>
<td>3.0 – 4.0</td>
<td>0.006*</td>
</tr>
<tr>
<td>Median</td>
<td>3.0</td>
<td>4.0</td>
<td>4.0</td>
<td></td>
</tr>
<tr>
<td>% of Change Baseline</td>
<td>134.26</td>
<td>↑26.85</td>
<td></td>
<td></td>
</tr>
<tr>
<td>% of Change</td>
<td></td>
<td></td>
<td>14.44</td>
<td></td>
</tr>
<tr>
<td><strong>Control group (n=9)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean ± SD.</td>
<td>3.44 ± 1.01</td>
<td>3.22 ± 1.09</td>
<td>3.11 ± 0.93</td>
<td>0.595</td>
</tr>
<tr>
<td>Min. – Max.</td>
<td>3.0 – 6.0</td>
<td>2.0 – 5.0</td>
<td>2.0 – 4.0</td>
<td>0.009*</td>
</tr>
<tr>
<td>Median</td>
<td>3.0</td>
<td>3.0</td>
<td>3.0</td>
<td></td>
</tr>
<tr>
<td>% of Change Baseline</td>
<td>7.28</td>
<td>↓6.48</td>
<td>12.22</td>
<td></td>
</tr>
<tr>
<td>% of Change</td>
<td></td>
<td></td>
<td>16.48</td>
<td></td>
</tr>
<tr>
<td>P</td>
<td>0.874</td>
<td>0.180</td>
<td>0.184</td>
<td></td>
</tr>
<tr>
<td>p: p value for Mann Whitney test for comparing between the two groups in each period</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>p_t: p for Friedman test for comparing between the three periods in each group</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

p: p value for Mann Whitney test for comparing between the two groups in each period
p_t: p for Friedman test for comparing between the three periods in each group

Concerning the study group, there was an increase in thickness of the keratinized mucosa from baseline to one month (from a mean of 3.56±1.59 to a mean of 3.89±0.78) and a decrease in thickness from one month to three months (from a mean of 3.89±0.78 to a mean of 3.67±0.50). However, there was no statistical significance difference between all studied periods with respect to this variable.
Table (3): Descriptive analysis of the studied cases according to healing score in two groups.

<table>
<thead>
<tr>
<th>Healing score</th>
<th>7 days</th>
<th>14 days</th>
<th>30 days</th>
<th>90 days</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Test group</strong> (n= 9)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Min. – Max.</td>
<td>3.0 – 3.0</td>
<td>1.0 – 3.0</td>
<td>0.0 – 1.0</td>
<td>0.0 – 0.0</td>
<td>&lt;0.01*</td>
</tr>
<tr>
<td>Mean ± SD.</td>
<td>3.0 ± 0.0</td>
<td>2.0 ± 0.71</td>
<td>0.56 ± 0.53</td>
<td>0.0 ± 0.0</td>
<td></td>
</tr>
<tr>
<td>Median</td>
<td>3.0</td>
<td>2.0</td>
<td>1.0</td>
<td>0.0</td>
<td></td>
</tr>
<tr>
<td>Sig.</td>
<td></td>
<td>p2=0.297</td>
<td>p3=0.002</td>
<td>p4=0.001</td>
<td>p5=0.264</td>
</tr>
<tr>
<td></td>
<td>&lt;0.001*</td>
<td>p6=0.037</td>
<td>p7=0.001</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Control group</strong> (n=9)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Min. – Max.</td>
<td>2.0 – 3.0</td>
<td>1.0 – 3.0</td>
<td>0.0 – 1.0</td>
<td>0.0 – 0.0</td>
<td>&lt;0.01*</td>
</tr>
<tr>
<td>Mean ± SD.</td>
<td>2.78 ± 0.44</td>
<td>2.22 ± 0.83</td>
<td>0.78 ± 0.44</td>
<td>0.0 ± 0.0</td>
<td></td>
</tr>
<tr>
<td>Median</td>
<td>3.0</td>
<td>2.0</td>
<td>1.0</td>
<td>0.0</td>
<td></td>
</tr>
<tr>
<td>Sig.</td>
<td></td>
<td>p2=0.456</td>
<td>p3=0.007</td>
<td>p4=0.001</td>
<td>p5=0.118</td>
</tr>
<tr>
<td></td>
<td>&lt;0.001*</td>
<td>p6=0.053</td>
<td>p7=0.001</td>
<td></td>
<td></td>
</tr>
<tr>
<td>P</td>
<td>0.145</td>
<td>0.507</td>
<td>0.331</td>
<td>1.000</td>
<td></td>
</tr>
</tbody>
</table>

p: p values for Mann Whitney test for comparing between the two groups in each period
p2: p for Friedman test for comparing between the three periods in each group, Sig. bet. Periods was done using Post Hoc Test (Dunn's multiple comparisons test)
p3: p value for comparing between 7 days and 14 days in each group
p4: p value for comparing between 7 days and 30 days in each group
p5: p value for comparing between 7 days and 90 days in each group
p6: p value for comparing between 14 days and 30 days in each group
p7: p value for comparing between 14 days and 90 days in each group
p8: p value for comparing between 30 days and 90 days in each group
*: Statistically significant at p ≤ 0.05

**Histological Evaluation**

Concerning the control group, the epithelium was parakeratinized with long epithelial ridges. The collagen fibers in the deep layer of the lamina propria were less dense, not running in bundles and having undefined orientation than those in the study group. Less inflammatory cells were noted than in the study group (Figure 5).

Regarding the study group, parakeratinized epithelium facing the oral cavity with long epithelial ridges seen extending deep into the underlying lamina propria. The superficial papillary layer of the lamina propria contained thin loosely arranged fibers while in the deep reticular layer the collagen fibers were arranged in thick bundles showing infiltration of inflammatory cells (Figure 6).

**DISCUSSION**

The outcome of placing dental implants immediately into fresh extraction sockets has been reported to be as predictable as placing implants into healed sites (17). However, achieving acceptable gingival esthetics around anterior single implants and maintaining it over time can be a challenging procedure. Maintaining the integrity of the mucogingival line and avoidance of any discrepancy in relation to adjacent teeth has become a demanding task, especially when restoring teeth with previous gingival recession and/or absence of attached gingival (18).

Connective tissue graft is considered the gold standard for soft tissue coverage over immediate dental implants.
Suresh and Gupta (30) also reported the ACM to be efficient in addition to laminins and fibronectins (31). KMT which continued to decrease to 6.48% at 3 months. That accounted for 34% increase in thickness at 1 month and decreased to a 26% increase at 3 months. On the other hand, the KMT was realized in the group treated with the ACM (within our clinical expectations) and all patients were fully satisfied with the esthetic results. The mucogingival junction remained stable in both groups relative to the adjacent teeth. The decrease in the KMW (mild recession) in the test and control groups may be attributed to the fact that implants placed immediately into fresh extraction sockets usually exhibit a degree of marginal bone loss due to alveolar socket remodeling. Thin buccal plates are usually more susceptible to the adverse effects of marginal bone loss and soft tissue recession (32). Chen et al (33) demonstrated that sites with thinner facial bone underwent significantly more vertical resorption than sites with thicker facial bone. Several studies have been reported on the frequency of recession at immediate implant sites. In a controlled study comparing immediate implants with implants placed 12 weeks after extraction, recession of 1 mm was found in seven of 23 immediate sites, compared to four of 25 12-week healed sites. Recession of 1 to 2 mm was observed in two of 23 immediate sites, but not in the 12-week healed group (34).

Ghahroudi et al (29) reported a mean increase in keratinized gingival width by 0.68±0.366 mm and 0.95±0.333 mm with ACM and subepithelial connective tissue graft groups, respectively in covering denuded roots. However, Oates et al (35) and Cordoni et al (36) emphasized the positive role of the subepithelial connective tissue graft in increasing the keratinized gingival width attributing to its ability to induce epithelial cell differentiation at the recipient site. Regarding the ACM, the presence of keratinocyte growth factor promotes keratinization of epithelial cells and helps the mucogingival junction to maintain its position, which explains the efficacy of the ACM in inducing keratinization. Since the positive role of both ACM and connective tissue graft in inducing keratinization and increasing the width of the keratinized gingiva is well documented, the decrease noticed in the current study may be attributed to the marginal bone loss after immediate implant placement.

Histological evaluation of soft tissue samples revealed adequate wound healing with mature tissue for both the control and the test groups. Samples showed varying degrees of maturation, connective tissue thickness, inflammation and vascularization mostly in favor of the ACM group.

The oral mucosa of the study group showed parakeratinized gingival epithelium; this finding is in agreement with Albana (2013) (24) who reported that the oral mucosa consists of the same normal epithelial structure. Concerning the control group, our examination of the oral mucosa showed a parakeratinized gingival epithelium, keratinized gingival epithelium that consisted of stratified squamous epithelium. The keratinized pattern represents a protective layer to the underlying epithelium. The epithelium showed, epithelial rete pegs, with loose connective tissue papillae running to a long distance in the alveolar mucosa near to the keratinous surface increasing nutrition and blood supply to the epithelium. These findings are consistent to those by Lewis et al in 2005 (37).

Maturation and proliferation of the oral epithelium are inline with the increase in loads and function that are applied on to it. Studies reported high rate of turnover of the cells of the oral epithelium, the cells undergoing mitosis in the basal layer and eventually migrating to the free surface. The basal cells move away from the basal layer perpendicularly and towards the surface of the epithelium to be desquamated (38).

In the ACM group the connective tissue showed densely arranged thick bundles of collagen fibers in the reticular...
layer for the mucoperiosteal junction making the gingival mucosa immobile and firm. Similar findings were reported by Laugerette et al (39).

In both groups, inflammatory cell infiltration was observed in the connective tissue. Inflammatory cells continually migrate into and pass between the epithelial cells. This was mentioned by Schroeder, 2012 who reported that the connective tissue supporting the epithelium, even in clinically normal circumstances, shows an inflammatory cell population such as polymorphonuclear leukocytes and T-lymphocytes (38).

Numerous blood vessels were observed distributed in the underlying connective tissue. This marked increase in blood supply in this region could be considered as a response to the ACM’s role in regulation of angiogenesis due to the presence of Laminin-5 (8). This increase in the blood supply suggests marked increase in the metabolic activity which probably led to active cell proliferation and high rate of turnover (40).

CONCLUSION
The present study proved that the use of ACM was very effective in covering immediately placed dental implants and enhancing wound healing. The ACM offered comparable results to the connective tissue graft concerning mucosal width, with better results regarding the mucosal thickness, healing and angiogenesis. Thus, it was concluded that ACM can be a valid alternative to connective tissue grafts for covering immediate dental implants, preserving the width of the keratinized mucosa and maintaining the integrity of the muco gingival line.

CONFLICT OF INTEREST
The authors declare that they have no conflicts of interest.

REFERENCES


38. Schroeder HE. Biological structure of the normal giniva– preface. Department of Periodontology, Faculty of Odontology, University of Lund, Malmo, Sweden and Department or Oral Structural Biology, Dental Institute, University of Zurich, Switzerland. Periodontol 2000 2012; 13: 7.
