Efficacy of Acellular Dermal Matrix and Coronally Advanced Flaps for the Treatment of Induced Gingival Recession Defects: A Histomorphometric Study in Dogs

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Background: Gingival recession (GR) defects can be treated by various methods, including acellular dermal matrix (ADM) or coronally advanced flaps (CAFs). The aim of this histomorphometric experiment is to compare the efficacy of ADM and CAF for treating GR defects in dogs.

Methods: In eight beagle dogs, a critical-size labial GR defect was surgically induced on bilateral maxillary cuspids under general anesthesia. Test sites received ADM and CAF, and control sites underwent CAF treatment alone. Plaque index (PI), bleeding index (BI), and gingival index (GI) were measured at 4 weeks (baseline), 8 weeks, and 16 weeks. Width of keratinized gingiva (KG) was determined at baseline and at 16 weeks. Depth of recession and width of GR below the cemento-enamel junction (CEJ) was also determined. After 4 months, animals were sacrificed, and jaw blocks were histomorphometrically assessed for tissue thickness and distance from the stent to the gingival margin (GM) and to the CEJ.

Results: At 4-, 8-, and 16-week intervals, there was no significant difference in the BI, GI, and PI at the test and control sites. At 16 weeks, thickness of KG was significantly higher at the control sites than test sites (P <0.01). There was no difference in the midfacial recession depth and recession width at the test and control sites at baseline and before euthanasia (16 weeks). Histomorphometrically, there was no significant difference in tissue thicknesses and distances from the stent to the GM and CEJ in the test and control sites.

Conclusion: ADM might yield similar results to a CAF alone and could decrease the amount of KG. J Periodontol 2013;84:1172-1179.

KEY WORDS
Acellular dermal matrix; connective tissue; gingival recession; periodontal diseases; surgical flaps; tissues.

Various treatment strategies have been proposed for the treatment of gingival recession (GR) defects.1,2 One of the earliest techniques used for root coverage was the thin free gingival graft (FGG) technique.3-5 The thin FGG was later modified as the thick FGG; however, the efficacy for root coverage remained dubious.6,7 Although this technique showed acceptable outcomes in shallow recession defects, the overall procedure was a failure.6,7 Nearly three decades ago, the connective tissue graft (CTG) was introduced for ridge augmentation and then for root coverage.8,9 Using CTG, successful GR defect coverage may be achieved with less donor tissue because revascularization occurs from both the periosteal or osseous base and the overlying flap.7,8 Coronally advanced flaps (CAFs) and their modifications have also been used for treating recession defects.10-12 Compared to other root coverage procedures, CAFs provide a better contour and color match to the site and do not require a donor site.10 However, lack of height and thickness of gingiva apical to the recession may limit the use of CAFs. Simultaneously, GR treated with
traditional flap designs such as the lateral sliding flap facilitate only a limited amount of bone and cementum formation.\textsuperscript{13}

Acellular dermal matrix (ADM) is harvested from human dermis and processed to remove all cellular and epidermal components. It maintains its structural framework with intact proteins, collagen fibrillar network, elastin filaments, hyaluronan and proteoglycans, and basement membrane, thus being able to serve as a soft tissue graft.\textsuperscript{14} ADM has been indicated for root coverage procedures and soft tissue as well as ridge augmentation.\textsuperscript{15,16} Unlimited supply and a less invasive surgery are the benefits of using ADM; however, its drawbacks include the lack of creeping attachment, healing by connective tissue adherence, and a need for adequate blood supply to facilitate graft integration.\textsuperscript{17-19} Successful treatment outcomes in terms of root coverage or gain of keratinized tissue have been documented over time.\textsuperscript{20,21} However, in terms of root coverage or gain of keratinized tissue, flap tension,\textsuperscript{28} and root surface preparation may influence root coverage protocols. These factors may be classified as anatomic, patient-related, and surgical factors. Anatomic factors that influence root coverage procedures include height of adjacent bone, dimensions of the adjacent interdental papilla, size of the defect, and flap thickness.\textsuperscript{22-26} Surgical factors, including operators’ clinical skills,\textsuperscript{27} flap tension,\textsuperscript{28} and root surface preparation techniques,\textsuperscript{29} have been demonstrated to influence outcomes of root coverage procedures. With regard to the regeneration of periodontal tissues, newly formed connective tissue attachment with collagen fibers inserting into cementum and bone are favored. This may occur provided cells, such as periodontal ligament cells, osteoprogenitor cells, and/or cementoblasts, are able to proliferate over the denuded root surface.\textsuperscript{30} It has been reported that ADM maintains space to allow for angiogenesis and tissue remodeling and increases the volume of attached gingiva and connective tissue.\textsuperscript{31} Clinical results by Aichelmann-Reidy et al.\textsuperscript{31} demonstrated that ADM may be a useful substitute for autogenous connective tissue grafts in root coverage procedures. The authors therefore hypothesized that root coverage using ADM is more effective in treating GR defects compared to traditional CAF regimens.

The purpose of the present histomorphometric study is to compare the efficacy of ADM and traditional CAF procedures for treating GR defects.

**MATERIALS AND METHODS**

**Ethical Guidelines**

The study was approved by the research ethics review committee of the Engineer Abdullah Bugshan Research Chair for Growth Factors and Bone Regeneration, 3D Imaging and Biomechanical Laboratory, College of Applied Medical Sciences, King Saud University, Riyadh, Saudi, Arabia. This study was also performed in accordance with the guidelines approved by the Council of the American Psychological Society for the use of animal experiments.

**Study Animals**

Eight female beagle dogs (mean age: 19 ± 1 months; mean weight: 13.8 ± 1 kg) were used. All dogs were prevaccinated against rabies and infectious hepatitis. The dogs were kept in individual cages and on a soft diet throughout the study period.

**Study Protocol**

All non-surgical and surgical procedures were performed under general anesthesia using intramuscular (IM) injections of ketamine\textsuperscript{¶} (10 mg/kg body weight) and local anesthesia with xylocaine (with epinephrine 5 mg/mL). IM antibiotics** (25 mg/kg body weight) were administered a day before and at the time of surgery. In all dogs, full-mouth scaling and root planing (SRP) was performed using an ultrasonic scaler\textsuperscript{††} and hand curets.\textsuperscript{‡‡} Removal of the keratinized tissue and full- and partial-thickness flap elevation were carried out using a sterile surgical blade #15 with two releasing incisions on the mesial and distal sides (Figs. 1A through 1C). A critical-size labial gingival recession defect (5 mm from the cemento-enamel junction [CEJ] and 5 mm mesial to distal) was surgically induced on both maxillary cuspids (Fig. 1D) by water-cooled sterile diamond and carbide burs and chisels. Hand instruments\textsuperscript{§§} were used to remove cementum from the root surface. SRP was performed on the root surface using an ultrasonic scaler and hand curets. As reference points, coronal (along the CEJ) and apical notches (at the level of alveolar crest) were made using a round diamond bur (Fig. 1D).

In each dog, two maxillary cuspids were randomly divided into two groups by picking a paper from a brown bag marked either “test site” or “control site.” Control sites were treated by CAF alone (Figs. 1E and 1F). Test sites received placement of an ADM membrane\textsuperscript{¶¶} in addition to CAF (Figs. 1G

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\textsuperscript{¶} Pfizer, Sandwich, Kent, UK.

\textsuperscript{¶} AstraZeneca for Dentsply Pharmaceutical, York, PA.

\textsuperscript{**} NorBrook Laboratories, Newry, Down, UK.

\textsuperscript{††} NSK, Westborough, MA.

\textsuperscript{‡‡} Hu-Friedy, Chicago, IL.

\textsuperscript{§§} NSK.

\textsuperscript{¶¶} Dermis, Zimmer Dental, Carlsbad, CA.
and 1H). In the test sites, the ADM membrane was trimmed and extended beyond the defect by 3 to 4 mm. Resorbable sutures§§ were used to secure the membrane to the tooth surface. In the test and control sites, flaps were passively positioned to cover the defect and primary closure was achieved using interrupted resorbable sutures. Postoperatively, antibiotics## (25 to 50 mg/kg IM every 8 hours) were continued for 5 days. All dogs received full-mouth SRP and application of 0.12% chlorhexidine*** via sterile cotton buds for 4 weeks. Sutures were removed 2 weeks after surgery.

Clinical Parameters
In the test and control sites, plaque index (PI), gingival index (GI), and bleeding index (BI) were measured at 4, 8, and 16 weeks. The following parameters were assessed: 1) width of keratinized gingiva (KG) at baseline right after surgery (considered as zero time) and at 4 and 16 weeks; 2) distance from stent to the gingival margin (GM); 3) distance from stent to CEJ; 4) depth of midfacial recession; and 5) width of GR below the CEJ.

Euthanasia and Histomorphometric Assessment
After 4 months of treatment, all dogs were sacrificed by an overdose of 3% sodium pentobarbital. All the blocks were washed in saline solution and immediately fixed in 10% formalin. They were then processed to obtain thin ground sections. The specimens were dehydrated in an ascending series of alcohol rinses and embedded in a glycolmethacrylate resin. After polymerization, the specimens were sectioned along their longitudinal axis with a high-precision diamond disk at about 150 μm and ground down to about 30 μm in the buccolingual plane at 0.1-mm intervals. Per specimen, three sections situated at the central region of the gingival recession were prepared that identified the coronal and apical notches. The slides were stained with acid fuchsin and toluidine blue. The slides were observed in normal transmitted light under a microscope and polarized-light microscopy. Histologic and histomorphometric analysis were performed using a microscope connected to a high-resolution video camera and software program. This optical system was associated with a digitizing pad and a histometry software package with image capturing capabilities.

A single investigator (GI) masked to the samples from the test and control sites collected the data. The histomorphometric measurements were 1) distance from the CEJ to the GMs and 2) buccolingual tissue thickness at two points, that is, 2 mm below the GM and at the coronal part of the stent (Fig. 2).

Statistical Analyses
Statistical analyses were performed using statistical software. The paired t test was used to compare the data.

Figure 1.
A through C) Removal of the keratinized tissue and elevation of full- and partial-thickness flaps. D) Placement of coronal (along the CEJ) and apical notches (at the level of alveolar crest). E and F) Control sites being treated by CAF alone and sutured. G and H) Test sites being treated by placement of a resorbable collagen membrane in addition to CAF and sutured.
assess the effect of the treatments, guided tissue regeneration–based root coverage, and CAF in the test and control group respectively. Data were expressed in millimeters as mean ± SE values. *P* < 0.05 was considered statistically significant.

**RESULTS**

**Clinical Parameters**

PI, BI, and GI at 4-, 8-, and 16-week intervals. At 4-, 8-, and 16-week intervals, there was no statistically significant difference in the mean scores of GI, BI, and PI at the test and control sites (Table 1).

**Width of keratinized gingiva.** At 4 weeks, there was no significant difference in the width of KG in the test and control sites. At 16 weeks, width of KG was significantly higher in the control sites compared to the test sites (*P* <0.01; Table 2).

**Histomorphometric Analyses**

Tissue thickness and distances from the base of notch to GM and to the CEJ. There was no statistically significant difference in the mean tissue thickness, distance from the base of the notch to the GM, and distance from the GM to the CEJ at the test and control sites (Table 2).

**Tissue morphology.** At low-power magnification, gingival mucosa constituted by oral epithelium (OE) and lamina propria (LP) was observed in the control sites. OE showed different degrees of keratinization, especially on the vestibular side. In the LP, small vessels can be detected. The gingival connective tissue was in contact with the root cementum and the notch (N in Fig. 3A), where a small gap (arrow in Fig. 3B) only in the most coronal portion can be observed. Small blood vessels and rare lymphocytes can also be seen (Fig. 4B).

**DISCUSSION**

The present results showed no significant differences between the test and control sites with reference to PI, GI, BI, tissue thickness of KG, and distances from the base of the notch to the GM and to the CEJ. These results are in accordance with...
previous studies,\textsuperscript{20,21} which showed that CAF and ADM can be successfully used for the treatment of GR defects. Similar results were also reported when collagen membrane was used as the graft material.\textsuperscript{34} The authors’ results are in agreement with these findings.

Owens and Yukna\textsuperscript{35} investigated the resorption rates of various barrier membranes in the oral cavity of dogs. The results demonstrated that at 1 month, all membranes showed slight-to-moderate degradation; at 2 months, all membranes had moderate-to-severe degradation; whereas at 3 months, all membrane types had severe degradation to not identifiable.\textsuperscript{36} It is therefore tempting to speculate that early degradation of ADM membrane in the present study may be responsible for yielding outcomes similar to the control group.

The present results showed no statistically significant difference in the clinical (PI, BI, and GI) and histomorphometric parameters between the test and control sites at 4-, 8-, and 16-week intervals. It is tempting to speculate that early resorption of ADM (within the first month of placement) may have inhibited new tissue-forming cells

### Table 1.

<table>
<thead>
<tr>
<th>Time Interval and Site</th>
<th>GI (mean ± SE)</th>
<th>BI (mean ± SE)</th>
<th>PI (mean ± SE)</th>
</tr>
</thead>
<tbody>
<tr>
<td>At 4 weeks</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Test sites</td>
<td>0.75 ± 0.16</td>
<td>1.00 ± 0.26</td>
<td>0.87 ± 0.22</td>
</tr>
<tr>
<td>Control sites</td>
<td>0.50 ± 0.18</td>
<td>1.00 ± 0.26</td>
<td>0.75 ± 0.25</td>
</tr>
<tr>
<td>At 8 weeks</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Test sites</td>
<td>2.250 ± 0.31</td>
<td>1.62 ± 0.31</td>
<td>0.00 ± 0.00</td>
</tr>
<tr>
<td>Control sites</td>
<td>1.375 ± 0.32</td>
<td>1.25 ± 0.36</td>
<td>0.00 ± 0.00</td>
</tr>
<tr>
<td>At 16 weeks</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Test sites</td>
<td>0.500 ± 0.26</td>
<td>0.875 ± 0.22</td>
<td>0.125 ± 0.12</td>
</tr>
<tr>
<td>Control sites</td>
<td>0.500 ± 0.26</td>
<td>0.500 ± 0.26</td>
<td>0.00 ± 0.00</td>
</tr>
</tbody>
</table>

* \( P < 0.01. \)

### Table 2.

<table>
<thead>
<tr>
<th>Time Interval and Site</th>
<th>Width of KG (mm; mean ± SE)</th>
<th>Stent to GM (mm; mean ± SE)</th>
<th>Stent to CEJ (mm; mean ± SE)</th>
<th>Midfacial Recession Depth (mm; mean ± SE)</th>
<th>Recession Width Below CEJ (mm; mean ± SE)</th>
</tr>
</thead>
<tbody>
<tr>
<td>4 weeks</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Test sites</td>
<td>4.14 ± 0.38</td>
<td>3.07 ± 0.44</td>
<td>4.14 ± 0.67</td>
<td>3.28 ± 0.32</td>
<td>8.57 ± 0.48</td>
</tr>
<tr>
<td>Control sites</td>
<td>4.35 ± 0.32</td>
<td>3.50 ± 0.42</td>
<td>4.21 ± 0.53</td>
<td>3.28 ± 0.28</td>
<td>8.00 ± 0.57</td>
</tr>
<tr>
<td>16 weeks</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Test sites</td>
<td>2.35 ± 0.34*</td>
<td>4.78 ± 0.88</td>
<td>4.00 ± 0.91</td>
<td>0.64 ± 0.26</td>
<td>3.07 ± 0.81</td>
</tr>
<tr>
<td>Control sites</td>
<td>4.64 ± 0.54*</td>
<td>3.78 ± 0.39</td>
<td>2.50 ± 0.95</td>
<td>0.28 ± 0.14</td>
<td>1.78 ± 0.97</td>
</tr>
</tbody>
</table>

* \( P < 0.01. \)

### Table 3.

<table>
<thead>
<tr>
<th>Histomorphometric Parameters</th>
<th>Test Sites (( \mu \text{m} ; \text{mean ± SE} ))</th>
<th>Control Sites (( \mu \text{m} ; \text{mean ± SE} ))</th>
<th>( P ) Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tissue thickness</td>
<td>758.77 ± 178.43</td>
<td>827.85 ± 77.89</td>
<td>0.47</td>
</tr>
<tr>
<td>Distance from the base of notch to GM</td>
<td>2.517.28 ± 719.43</td>
<td>4.160.2 ± 641.2</td>
<td>0.44</td>
</tr>
<tr>
<td>Distance from GM to CEJ</td>
<td>474.3 ± 650.39</td>
<td>271.37 ± 701.76</td>
<td>0.22</td>
</tr>
</tbody>
</table>
from populating at the test sites, thereby yielding results similar to those of the control sites. This may also be an explanation for the histomorphometric results that showed width of KG to be significantly greater at the control sites compared to the test sites at 4 months in the present study. These results contradict previous studies,15,16 which showed that root coverage with ADM increased thickness of KG compared to root coverage performed without using ADM. These studies15,16 propose that the presence of collagen fibers in the ADM membrane facilitates root coverage by stimulating platelet attachment, enhancing fibrin linkage, and having a chemotactic effect on fibroblasts.16 However, in a recent systematic review, Hoffmänner et al.36 indicated that in Miller Class I and II recessions, root coverage by CAF can yield predictable outcomes, and the use of bioabsorbable membranes with CAF is effective in Miller Class III recessions. The results of the present study also show no significant difference in tissue thicknesses and distances from the notch to the GM and CEJ in the test and control sites. This probably can be explained by the thick tissue biotype noted in this study, which diminished the ability of ADM to increase tissue thickness. Another possible explanation is the high resorbable rate noted with ADM as reported by others.37-40 Studies by Wei et al.38,39 showed ≥30% to 40% shrinkage, and Yan et al.37 reported 78% shrinkage at 3 months that became 82% at 6 months. Harris40 also demonstrated a significant ADM shrinkage at 4 years of follow-up. Interestingly, some studies37-41 have reported that although both CTG and ADM increase the gingival thickness, a greater improvement occurs in sites treated with CTG. However, other experimental studies42,43 that compare CAF with or without ADM in treatment of GRs showed successful outcomes in gaining gingival thickness with the adjunctive use of ADM.

Studies10,29 have reported that the size of the induced defect may influence the outcome of root coverage protocols. Pini-Prato et al.29 reported that root coverage is greater when GR is >4.98 mm. In the present experiment, size of the defect is in accordance with that recommended by the Pini-Prato study29; however, no significant influence of defect size on root coverage is observed. Further studies regarding the influence of GR size on root coverage are required.

It is well known that systemic conditions (such as poorly controlled diabetes and impaired glucose

![Figure 3. Histomorphometric results at the control site.](image)

**A)** Different degrees of keratinization can be detected in the oral epithelium. The notch (N) can be seen. (Original magnification ×12.) **B)** A small gap (arrow) is present in the coronal portion of the notch. (Original magnification ×40.) **C)** Low-grade inflammatory infiltrate. (Original magnification ×100.)

![Figure 4. Histomorphometric results at the test site.](image)

**A)** Low-power magnification of gingival mucosa, constituted by OE and LP, which is in contact with dentin (D) at the notch level. (Original magnification ×12.) **B)** Small blood vessels (*) and rare lymphocytes. (Original magnification ×40.) **C)** Inflammatory exudate and blood vessels (*). (Original magnification ×100.)
tolerance) and tobacco habits (e.g., cigarette smoking) jeopardize periodontal healing.\textsuperscript{44-46} It is hypothesized that the clinical outcomes of ADM and CAF treatments may vary among individuals who are immunocompromised and habitual consumers of tobacco; however, further studies are warranted in this regard. Furthermore, in the present experiment, tissue regeneration was attempted in the absence of any adjunctive biomimicry therapies. Recent studies\textsuperscript{47-49} have reported that biomimicry agent (such as enamel matrix derivative [EMD]) proteins promote tissue repair and regeneration at defected sites. These studies\textsuperscript{47-49} revealed that EMD proteins exhibit the potential to enhance the healing of standardized epithelial wounds, induce cementogenesis, and increase the thickness of gingival tissues. It is therefore hypothesized that treatment of GR defects using ADM with adjunct EMD therapy may enhance gingival tissue thickness and yield positive outcomes in terms of root coverage. However, it remains to be determined whether these surgical procedures under strict oral hygiene measures would be effective in the treatment of GR defects in chronic users of tobacco and alcohol.

**CONCLUSION**

Within the limits of the present experiment, it is concluded that ADM might yield similar results to CAF alone and could decrease the amount of KG.

**ACKNOWLEDGMENTS**

The authors would like to thank the College of Dentistry Research Center and the Deanship of Scientific Research at King Saud University, Saudi Arabia, for funding this research project (Research Project #NF 2380). The authors report no conflicts of interest related to this study.

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Submitted June 18, 2012; accepted for publication September 19, 2012.