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The Effect of EDTA in Attachment Gain and Root Coverage

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Abstract: Root surface biomodification using low pH agents such as citric acid and tetracycline has been proposed to enhance root coverage following connective tissue grafting. The authors hypothesized that root conditioning with neutral pH edetic acid would improve vertical recession depth, root surface coverage, pocket depth, and clinical attachment levels. Twenty teeth in 10 patients with Miller class I and II recession were treated with connective tissue grafting. The experimental sites received 24% edetic acid in sterile distilled water applied to the root surface for 2 minutes before grafting. Controls were pretreated with only sterile distilled water. Measurements were evaluated before surgery and 6 months after surgery. Analysis of variance was used to determine differences between experimental and control groups. We found significant postoperative improvements in vertical recession depth, root surface coverage, and clinical attachment levels in test and control groups, compared to postoperative data. Pocket depth differences were not significant (P<.01).

Gingival recession is an undesirable condition that results in root exposure. This condition is often unesthetic and may lead to sensitivity and root caries. Exposed root surfaces may also exhibit abrasion. Gingival recession is characterized by the displacement of the gingival margin apically from the cementoenamel junction (CEJ), or from the former location of the CEJ where restorations have distorted its location or appearance. Gingival recession can be localized or generalized and can be associated with one or more surfaces. The prevalence of 1 mm or more recession has been found in 58% of the US population.

One goal of soft-tissue grafting is to treat gingival recession by root coverage. Many techniques and flap designs have been used, some of which do not require a donor site (pedicle grafts), and others do (free autogenous grafts). It is sometimes difficult to anticipate the success rate of root-coverage procedures because root coverage may depend on several factors such as classification of the recession, the technique used, and the location of recession. The gingival dimension most commonly assessed is the height (distance between the free gingival margin and the mucogingival line measured in millimeters). An increase in gingival height independent of the number of millimeters is considered a successful outcome of augmentation procedures.

The use of connective tissue grafts for root coverage was reported by Langer and Langer. They used a partial thickness flap with 2 vertical incisions at the recipient site, followed by placement of a graft collected from the palate by a double-parallel incision technique. The flap is coronally positioned to cover the graft and benefit from a double blood supply. They reported an overall average increase of 2 mm to 6 mm of root coverage in 56 cases over 4 years. Borghetti and Louise, in their split mouth controlled clinical study, reported a 70% success rate of root coverage after connective tissue grafting. The results were obtained 1 year after surgery.
Clinicians in most studies using connective tissue grafts for root coverage did not attempt to remove the epithelial collar from the graft, but Bouchard and colleagues did in their study, and no additional benefits were observed. Harris compared parallel incisions and free gingival knife methods for donor collection of connective tissue grafts. He found that there was no difference in the percentage of root coverage with different techniques.

It is sometimes difficult to anticipate the success rate of root-coverage procedures because root coverage may depend on several factors.

In an attempt to increase the success rate of root coverage, many clinicians have tried a variety of techniques. Nelson combined connective tissue grafting with the double-pedicle graft. First, a free connective tissue graft was placed over the denuded root surface, then a double-pedicle graft was attempted to partially cover the connective tissue graft. The mean root coverage was 88% advanced recession (7 mm to 10 mm), 92% moderate recession (4 mm to 6 mm), and 100% slight recession (3 mm or less). Harris modified Nelson's technique by using a split-thickness pedicle graft to cover the connective tissue graft. He found mean root coverage of 97%. Wennström and Zucchelli compared a coronally positioned flap procedure to a combination coronally positioned and connective tissue graft procedure. The success rate for the combination group was 98.9%, while 97% was accomplished for the control group after an evaluation 2 years after surgery.

When using manual or ultrasonic instrumentation for planing the denuded root surface before the root-coverage procedure, the objective is to remove calculus and diseased cementum to create a clean root surface that will be biologically acceptable to promote cell growth and facilitate attachment formation. However, root planing creates a smear layer that adapts to the root surface and cannot be removed by conventional rinsing with water or saline solutions. Exposed root surfaces in patients with gingival recession may also display a hypermineralized surface layer and endotoxin contamination. To remove that smear layer, many clinicians are using root biomodification agents such as ethylenediaminetetraacetic acid (EDTA), tetracycline, or citric acid. The use of root biomodification agents has been studied in vivo and in vitro. Those studies showed that the chelating agent EDTA, acting at neutral pH, appears preferable for exposure of collagen fibers and early cell colonization. In addition, etching at neutral pH has been reported to preserve adjacent tissue vitality, while etching at low pH kills the flap and adjacent tissue after 20 seconds of exposure.

Clinical studies have also investigated the effect of EDTA on clinical parameters by examining smear layer formation following different root planing techniques. After examination by scanning electron microscopy, the results indicated that a smear layer formed upon root planing, and removal of that smear layer was accomplished by using 24% EDTA. The possibility of using EDTA as an irrigating agent in combination with ultrasonic scalers or in conjunction with mechanical root planing also has been suggested.

The authors of the previous study concluded that the smear layer removal and collagen fiber exposure was achieved when EDTA was applied through a customized tip, but no additional improvement in clinical parameters was observed. In a following study, they evaluated the effect of EDTA on clinical parameters after flap surgery to treat intraosseous defects. There was no additional benefit obtained by using EDTA. The effect of EDTA on smear removal and collagen exposure was also demonstrated using different concentrations. When concentrations of 1.5%, 5%, 15%, and 24% were applied for a duration of 2 minutes, only concentrations of 15% and 24% accomplished complete removal of the smear layer and exposure of collagen fibers.

Previous studies have evaluated the effect of EDTA in models of gingival inflammation. The purpose of this study was to determine the effect of root conditioning with neutral pH EDTA using a recession model of relative gingival health.
Materials and Methods

For this study, we used 10 systemically healthy nonsmokers (5 men and 5 women, ages 25 to 35) who had bilateral gingival recession on canines or premolars (Figure 1). Twenty total sites of recession, Miller class I and II, that required connective tissue grafting, were evaluated in the patients and presented to the postgraduate periodontics clinic at the University at Buffalo and the periodontics clinic at Marquette University. The study included 1 operator/examiner who treated 5 patients, totaling 10 sites of recession, at each clinic.

Only patients with good oral hygiene (plaque index <0.5)b were included in the study. Exclusion criteria included the presence of caries or restorations at the recession sites, teeth with occlusal trauma, or prior treatment with local chemotherapeutic agents. In addition, patients taking systemic medications or with medical conditions that contraindicated periodontal surgery were excluded. Patients who used tobacco products also were excluded from the study.

The recession sites were divided into 2 groups and randomly assigned by a computer program. One site was assigned to group A, and the contralateral site was assigned to group B.

The root conditioning agent and control were placed into 2 identical vials labeled control and test. The control vial contained sterile distilled water, and the test vial contained 24% EDTA in sterile distilled water. During the study, the operator/examiner was unaware of the components of the vials. After completing the study, the code was broken and the data were analyzed.

The procedures in this study were reviewed and approved by the institutional review boards of the Schools of Medicine and Biomedical Sciences and Dental Medicine at the University at Buffalo, and the Institutional Review Board, Office of Research Compliance at Marquette University. After signing a written informed consent, the recession sites were randomly assigned to either group A or B. All patients received oral hygiene instruction before surgery, at weeks 1 through 4, and then every 2 weeks for 6 months after surgery. Before surgery, a record of the defect was obtained by using an impression material (polyvinyl siloxane Type 0, very high consistency), and a stone model was fabricated.

Clinical attachment levels (CALs) were measured at the mesiobuccal, midbuccal, and distobuccal aspects of the tooth using the CEJ as a reference. Pocket depth (PD) measurements were collected using a manual periodontal probe (UNC, 15 mm probe) at the mesiobuccal, midbuccal, and distobuccal aspects of the tooth. The vertical dimension of recession (VR) was measured at the midbuccal aspect of the tooth from the free gingival margin to the CEJ. All clinical measurements were obtained at baseline and 6 months after surgery.

The percentage of root surface coverage (RSC) was calculated by using the prefabricated stone models collected at baseline and 6 months after surgery (Figure 2). Image Pro Plus® computer software was used to scan and calculate the before and after surface area of the recession. The percentage of RSC was calculated by subtracting the recession measurement before surgery from the measurement 6 months after surgery. The statistical analysis (t-test) was completed using computer software (SPSS 12.0.1®).
**Surgical Procedures**

The surgical procedures performed in this study were as described by Langer and Langer.

**Recipient Site**

Local infiltration anesthesia was obtained using 2% lidocaine HCl with epinephrine (1:100,000). Before flap reflection, root planing was performed for 5 minutes using hand curettes, followed by application of a root conditioning agent. Group A sites were conditioned with the control using a cotton applicator with light pressure and a rubbing motion for an additional 2 minutes. Group B sites were conditioned with the test and treated identically. A sulcular incision was made to preserve the existing radicular gingiva. A partial thickness flap subsequently was prepared by making 2 vertical releases (Figure 3). The interproximal papilla was left intact. After placement of the graft, the flap was coronally positioned to cover, and the area was sutured with 5-0 resorbable sutures (Figure 4). A light-cured periodontal dressing was placed to cover the grafted area.

**Donor Site**

Local infiltration anesthesia was obtained using 2% lidocaine HCl with epinephrine (1:100,000). A horizontal incision was made in the soft tissue, approximately 5 mm to 6 mm from the free gingival margins of the maxillary teeth near the premolar area using a parallel, double-blade scalpel, and was continued apically toward the alveolar bone to the desired length. Two internal vertical incisions were made on both sides of the horizontal incision to facilitate the removal of the connective tissue graft. A graft thickness of 1.5 mm typically was prepared. The graft was placed over the denuded root and sutured in place. The donor connective tissue was sutured to the underlying connective tissue using 4-0 resorbable sutures.

Chlorhexidine gluconate 0.12% mouthrinse was prescribed for all patients (twice daily rinses for the first month after surgery). For pain management, patients were advised to take 400 mg of ibuprofen every 4 to 6 hours as needed. Oral hygiene instructions and prophylaxis were also performed if needed at each visit after surgery.

**Results**

**Pocket Depth**

The mean PD included 2 measurements. The first measurement was taken at the mid-buccal surface (PD buccal), and the second measurement was the mean of 2 interproximal measurements on the buccal surface (PD interproximal). There were no statistically significant differences in either buccal or interproximal measurements from baseline to 6 months after surgery (P>.01, Table 1). In the test group, the mean PD buccal decreased from 1.7 mm to 1.6 mm while the PD interproximal measurement remained at 2.0 mm. In the control group, the mean PD buccal and interproximal measurements remained the same from before to after surgery (2.0 mm). There were no statistically significant differences between the 2 groups (P>.01, Table 2).

**Clinical Attachment Levels**

The mean CAL was calculated using the CEJ as a reference point coronally and the base
of the pocket apically. CAL included 2 measurements. The first measurement was taken at the midbuccal surface (CAL buccal), while the second measurement was the mean of 2 interproximal measurements on the buccal surface (CAL interproximal). There were statistically significant differences in only mean CAL buccal from baseline to 6 months after surgery (P<.01, Table 1). In the test group, the mean CAL buccal increased 4.1 mm, and the mean CAL interproximal increased 0.1 mm. In the control group, the mean CAL buccal increased 4.0 mm and the mean CAL interproximal increased 0.3 mm. There were no statistically significant differences between the groups (P>.01, Table 2).

**Vertical Recession Depth**

The mean VR was measured clinically at the midbuccal surface, from the CEJ to the free gingival margin (Figure 5). There was significant improvement in VR—from 4.2 mm before surgery to 0.1 mm after surgery (P<.01, Table 1). In the test group, the mean VR decreased from 4.3 mm to 0.1 mm. In the control group, the mean VR decreased from 4.0 mm to 0.1 mm. There were no statistically significant differences between the groups (P>.01, Table 2). The coverage percentage of vertical recession depth was 97.7% for the test group and 97.5% for the control group, which was not statistically significant (P>.01, Table 2).

**Root Surface Coverage**

The mean RSC was measured on a prefabricated stone model obtained at baseline and 6 months after surgery. The percentage of RSC was collected via computer software. Statistically significant differences in RSC were obtained after surgery with a mean increase from 16.7 mm to 0.46 mm (P<.01, Table 1). In the test group, the recession defect area decreased from 17.5 mm to 0.5 mm, and in the control group, the recession defect area decreased from 15.9 mm to 0.4 mm. The gain in RSC was not statistically significant between the groups (P>.01, Table 2). The coverage percentage of RSC for the test group was 97.1%, and for the control it was 97.5%, which was not statistically significant (P>.01, Table 2).
**Discussion**

Soft-tissue grafting can be used to treat gingival recession and achieve root coverage. Techniques and flap designs have been introduced to obtain root coverage, but connective tissue grafts in conjunction with coronally positioned flaps have been used by practitioners to achieve esthetic results when treating gingival recession. The results of this study confirmed that the combination of connective tissue grafting and coronally positioned flaps can be used successfully to treat gingival recession defects, with significant root coverage obtained 6 months after surgery.

The results achieved in the present study agree with those reported in the literature for coverage of localized gingival recession.\(6,7,10\) Recessions were reduced by an average of 4.1 mm (Table 1). Because there were minimal changes detected in pocket depth (0.1 mm reduction), the coverage achieved was the result of an increase in the clinical attachment levels. However, the type of attachment to the root surface could not be assessed in this study.

The smear layer has been described as a layer composed of particles of mineralized collagen matrix present after root planing.\(12\) Studies have suggested that the smear layer may serve as a barrier to the development of a connective tissue attachment to the root surface.\(12\) The hypothesis of using neutral pH agents as root conditioners included the removal of the smear layer and exposure of collagen fibers to obtain new connective tissue attachment. The use of such agents was pro-

<table>
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<tr>
<th>Clinical Parameters</th>
<th>EDTA (mm)</th>
<th>SD</th>
<th>N</th>
<th>Control (mm)</th>
<th>SD</th>
<th>N</th>
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<tbody>
<tr>
<td>PD Buccal (Preoperative)</td>
<td>1.7</td>
<td>0.5</td>
<td>10</td>
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<td>10</td>
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<td>PD Buccal (Postoperative)</td>
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<td>2.0</td>
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<td>10</td>
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<td>10</td>
<td>2.0</td>
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<tr>
<td>CAL Buccal (Preoperative)</td>
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<td>5.6</td>
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<td>1.6</td>
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<tr>
<td>CAL Interproximal (Preoperative)</td>
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<td>10</td>
<td>2.3</td>
<td>0.5</td>
<td>10</td>
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<tr>
<td>CAL Interproximal (Postoperative)</td>
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<td>2.0</td>
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<td>10</td>
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<td>10</td>
<td>4.0</td>
<td>0.5</td>
<td>10</td>
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<tr>
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<td>10</td>
<td>0.1</td>
<td>0.3</td>
<td>10</td>
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<td>Root Surface coverage (RSC) (Preoperative)</td>
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<td>1.9</td>
<td>10</td>
<td>15.9</td>
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<td>10</td>
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<tr>
<td>Root Surface coverage (RSC) (Postoperative)</td>
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<td>0.3</td>
<td>10</td>
<td>0.4</td>
<td>0.3</td>
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<tr>
<td>Coverage Percentage of (VR)</td>
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<td></td>
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<td>97.5</td>
<td></td>
<td>10</td>
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<tr>
<td>Coverage Percentage of (RSC)</td>
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<td></td>
<td>10</td>
<td>97.5</td>
<td></td>
<td>10</td>
</tr>
</tbody>
</table>

Data are reported in mm (means ± SD).
All comparisons were not statistically significant when compared to control (P > .01).
N: Number of patients.
SD: Standard deviation.
PD: Pocket depth.
CAL: Clinical attachment level.
posed after studies found that there are necrotizing effects and delays in healing at adjacent tissues because of using low pH agents. Although many animal studies yielded promising results, clinical studies with humans were disappointing. The reasons for the disparity in results are unclear, and further elucidation of the differences obtained with various experimental modalities may be limited by the relative difficulty in obtaining sufficient human materials for histological analysis.

Studies have suggested that the smear layer may serve as a barrier to the development of a connective tissue attachment to the root surface.

Human clinical studies have compared the effect of EDTA on a variety of clinical parameters. However, those studies generally have assessed inflamed tissue (e.g., flap surgery for pocket reduction and treatment of intrasosseous defects). There have been few studies that have evaluated EDTA as a root conditioner in a healthy environment (gingival recession). Previous clinical and animal studies have failed to find adverse effects when using 15% to 24% EDTA for 5 minutes or less as a root conditioner. In this study, no adverse effects were observed or reported when 24% EDTA was used as a root conditioner for 2 minutes. Further controlled clinical and histological studies should be performed to evaluate the nature of attachment after applying EDTA and to compare that with clinical parameters.

Conclusion

Within the limits of this study, it can be concluded that connective tissue grafting resulted in significant improvement after surgery in VR, RSC, and CAL in both test (EDTA) and control groups. Pocket depth differences were not significant (P<.01). However, the use of EDTA as a root conditioner did not provide any additional statistically significant benefit.

References


1. The prevalence of 1 mm or more recession has been found in what percent of the American population?
   a. 18%
   b. 78%
   c. 28%
   d. 58%

2. Pedicle grafts:
   a. do not require a donor site.
   b. have poor esthetic results.
   c. have poor blood supply.
   d. require a donor site.

3. Root coverage may depend on which factor?
   a. classification of recession
   b. technique used
   c. location of recession
   d. all of the above

4. Borghetti and Louise, in their split mouth controlled clinical study, reported what percent success rate of root coverage after connective tissue grafting?
   a. 20%-30%
   b. 50%-60%
   c. less than 10%
   d. 70%

5. When using manual or ultrasonic instrumentation for planing, the objective is to:
   a. remove the smear layer.
   b. remove calculus and diseased cementum.
   c. remove the remaining periodontal ligament and collagen attachment above the cementoenamel junction.
   d. remove cementum, dentin, and enamel.

6. What creates a smear layer that adapts to the root surface?
   a. use of a low pH root conditioning agent
   b. stabilization of the blood clot during the healing phase
   c. root planing
   d. use of a neutral pH root conditioning agent

7. The chelating agent EDTA acting at neutral pH:
   a. appears preferable for exposure of collagen fibers.
   b. appears preferable for early cell colonization.
   c. preserves adjacent tissue vitality.
   d. all of the above

8. Etching at low pH:
   a. causes instability of the blood clot.
   b. will eliminate all of the bacterial contamination on the root surface.
   c. kills the flap and adjacent tissue.
   d. is contraindicated after previous periodontal surgery.

9. Complete removal of the smear layer and exposure of collagen fibers was accomplished by using which concentrations of EDTA?
   a. 5% and 10%
   b. 15% and 24%
   c. 1% and 2%
   d. 70% and 80%

10. The hypothesis of using neutral pH agents as root conditioners included:
    a. removal of the smear layer.
    b. control bleeding.
    c. inhibit osteoblast activity.
    d. inhibit osteoclast activity.

Please see tester form on page 362.