# A COMPARATIVE STUDY OF ROOT COVERAGE USING DYNAMATRIX PLUS VERSUS CONNECTIVE TISSUE GRAFT

A Thesis

by

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#### ABSTRACT

The purpose of this randomized clinical study was to compare the root coverage outcomes and clinical attachment levels in areas of facial gingival recession between an autograft and a xenograft. The materials investigated were autogenous connective tissue graft (CTG) and porcine derived extracellular matrix (ECM, DynaMatrix Plus<sup>®</sup>, Keystone Dental).

Twenty-two non-smoking, healthy patients participated in the study based on their existing gingival recession. Patients with qualifying Miller Class I, II, or III gingival recession defects were evaluated and randomly assigned to either CTG (control) or ECM (test) groups. Patients that presented with two similar, bilateral defects had one defect treated with CTG and the other with ECM. Both test and control sites were treated with identical surgical technique with the only difference in treatment being the graft material. Clinical parameters included: root vertical recession (VR), horizontal recession, probing depth (PD), clinical attachment level (CAL), bleeding on probing, keratinized tissue width (KT), and papillary height and width. All patients had measurements taken at baseline, 3 and 6 months after surgery.

Two patients dropped out of the study. Thus 20 patients with 28 sites completed the study. Thirteen sites received CTG while 15 sites received ECM. Baseline mean VR (CTG =  $2.81\pm0.663$  mm and ECM =  $2.73\pm0.594$ ) and CAL (CTG =  $4.27\pm0.992$  mm and ECM =  $4.20\pm0.797$  mm) showed no significant difference between groups. At 6 months, mean VR decreased significantly in both groups (CTG =  $0.69\pm0.879$  mm and

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 $ECM = 0.97\pm0.694$  mm), whereas mean CAL increased significantly in both groups ( $CTG = 2.00\pm0.913$  mm and  $ECM = 2.00\pm0.732$  mm). Intergroup differences in mean VR and CAL were non-significant at 6 months. KT was higher in the test group than the control group at 6 months, although the intergroup difference was not statistically significant. The results of 20 Miller Class III defects of the total 28 sites were analyzed separately, and no statistically significant differences were detected in VR and CAL between test and control.

Based on the results of this study, VR and CAL improved significantly in both test and control groups from baseline to 6 months post-operatively. There was no significant long term (6 months) difference between test and control groups in VR and CAL.

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#### CHAPTER I

#### INTRODUCTION AND LITERATURE REVIEW

The scope of periodontics has broadened considerably in the past few decades with advances in the treatment of periodontitis, replacement of missing and hopeless teeth through dental implants, and periodontal plastic surgery. The specialty has more treatment options than ever. Before reviewing advances in treatment, it is important to appreciate the ground work that has been laid down by previous clinicians and researchers. To begin, the field of periodontics is defined as a "specialty of dentistry which encompasses the prevention, diagnosis and treatment of diseases of the supporting and surrounding tissues of the teeth or their substitutes and the maintenance of the health, function and esthetics of these structures and tissues."<sup>1</sup> The American Academy of Periodontology defined periodontal surgery as "any surgical procedure used to treat periodontal disease or to modify the morphology of the periodontium."<sup>2</sup> Broadly, periodontal surgery consists of three surgical treatments: resective, reparative, and regenerative. Often, the management and treatment of periodontal disease, replacement of missing teeth, and periodontal plastic surgery are accomplished through these three surgical categories. The purpose of this thesis is to review the advances of periodontal surgery, and focus on the history and current status of periodontal plastic surgery in the treatment of acquired mucogingival defects which includes gingival recession.

Resective surgery encompasses procedures like gingivectomy and osseous surgery. Gingivectomy, first described by Orban, consists of the excision of the soft

tissue encompassing the periodontal pocket in an effort to treat periodontal disease.<sup>3</sup> However, inconsistent results lead to the development of osseous surgery. The principles of modern day osseous surgery were pioneered by Schluger.<sup>4</sup> Schluger described that the recurrence of pockets following gingivectomy was due to the persistence of irregular bony contours beneath the soft tissues. According to Schluger, soft tissues can only tolerate a 30 degree variation in the alveolar bone and any bony contour greater than this should be reduced. Pivotal improvements in technique can be attributed to the work of Ochsenbein and Bohannan and their palatal approach to osseous surgery.<sup>5</sup> Removal of irregular bony contours that are frequently found at interproximal sites was followed by a reduction in attachment on primarily the palatal surface of the tooth in order to recreate positive architecture.<sup>5</sup>

Healing following a resective procedure, specifically gingivectomy, was studied by Engler *et al* in monkeys.<sup>6</sup> Epithelialization of the surgery site began at 12 to 24 hours following excision, and required 4 to 5 weeks until the healing was noted as completed. Furthermore, a fully epithelialized surface formed 2 weeks post-operatively. Engler *et al* noted that the epithelium migrated from wound margins at a rate of 0.5 mm per day. Listgarten studied post-operative healing in monkeys using electron microscopy and concluded that the junctional epithelium was reestablished completely as early as 12 days post-excision.<sup>7</sup> Similarly, Stahl *et al* found that epithelialization in monkeys and humans could be obtained at 7 to 14 days and that connective tissue maturity could be acquired at 10 to 30 days after gingivectomy.<sup>8</sup> Ramfjord found that healing following

gingivectomy in monkeys resulted in connective tissue formation 1 to 2 days postoperatively but required a full 3 to 5 weeks before reaching maturity.<sup>9</sup>

Reparative periodontal procedures include open flap debridement and the modified Widman flap. Sites treated by reparative procedures healed in a manner that does not fully restore the architecture or the function of the periodontium.<sup>10</sup> Becker *et al* studied the repair of three-wall intrabony defects following open flap debridement.<sup>11</sup> They classified the intrabony defects based upon width as narrow (1 to 2 mm), medium (2 to 3 mm), or wide (> 4 mm). Becker *et al* took impressions of the intrabony defect intra-operatively. Results of the study demonstrated a mean defect fill of 2.56 mm (61%) and an attachment gain of 3.26 mm. Specifically, narrow defects had the greatest amount of gain compared to moderate and wide defects.<sup>11</sup> The clinical findings by Becker *et al* support the use of reparative procedures to treat patients having intrabony defects.

Ramfjord and Nissle described the modified Widman flap.<sup>12</sup> They recommend it to minimalize soft tissue recession and to treat deep pockets. The procedure calls for 3 incisions to facilitate removal of the sulcular epithelium. The first incision is directed parallel to the long axis of the tooth and is placed 0.5 to 1 mm away from the free gingival margin so that all of the crevicular epithelium is included. A second incision is made from the bottom of the sulcus to the alveolar crest. The final incision is completed with an orban knife in the interproximal region towards the alveolar process so that the collar of tissue can be separated and removed. Removal of the tissue collar allows for access to the diseased root surface. After debridement, the gingival tissues are

repositioned to allow for a fresh connective tissue bed to lie intimately on the root surface. However, Smith *et al* found no benefit in outcome with the removal of the sulcular epithelium in the modified Widman flap procedure.<sup>13</sup>

The goal of regenerative therapy is to reproduce or reconstitute lost or injured periodontal structures. Specifically, regenerative therapy aims to create a new attachment between the tooth and the periodontium. New attachment is defined as, "The union of connective tissue or epithelium with a root surface that has been deprived of its original attachment apparatus. This new attachment may be epithelial adhesion and/or connective tissue adaptation or attachment and may include new cementum".<sup>10</sup> Methods to regenerate bone, periodontal ligament attachment, cementum, and connective tissue have been investigated by numerous researchers. Grafting of intrabony defects in the alveolus with autogenous iliac crest marrow was described by Schallhorn.<sup>14</sup> The overall mean bone fill of all intrabony defects was 3.33 mm. Complete fill of intrabony defects favored 2-walled defects over 1-wall and no-wall defects. Class II furcations also showed favorable results of complete bone fill. However, the disadvantage with iliac crest grafts includes root resorption, infection, and sequestration.<sup>15</sup>

Autogenous harvesting of fresh iliac bone evolved into the era of freeze-dried bone allografts. Bowers evaluated new attachment of exposed tooth root surfaces associated with intrabony defects in humans.<sup>16</sup> Teeth that were debrided and then had their coronal portion resected so that the tooth roots were submerged below the gingiva demonstrated more gain in new attachment. However, the study demonstrated that a non-submerged tooth with a demineralized freeze-dried bone allograft also achieved

some level of new attachment. Since patient acceptance and more importantly tooth functionality would be sacrificed with the former treatment option, only the latter could be applied in a clinical setting.

Mucogingival surgery is utilized to correct deformities in the shape, amount, and location of gingiva. Mucogingival surgery uses a combination of the principles utilized in resective, reparative, and regenerative surgical categories. Examples of mucogingival surgery include but are not exclusive to the following: removal of aberrant frenum attachment, augmenting keratinized tissue width, root coverage, augmentation of edentulous ridge thickness, and coronal positioning of a gingival flap.

Gingival recession is a prevalent condition that affects a large portion of the adult population. In a study by Marini *et al*, 380 adult subjects were randomly selected from Bauru Dental School in Sao Paulo, Brazil.<sup>17</sup> Marini *et al* found that 89% of the individuals selected for this study exhibited at least one dental surface with gingival recession. Gingival recession was defined as 1 mm or more of root surface exposure in the vertical dimension from the cementoenamel junction to the gingival margin. Specifically, 3,526 teeth and 6,123 surfaces had gingival recession. The majority of these defects were Miller Class I and III defects, (59% and 33% respectively). Furthermore, age and severity of gingival recession were strongly correlated, i.e. with age the vertical dimension of the defect tended to increase. Miller class III defects increased with age while Miller class I defects decreased with age. In addition, gingival recession affected the mandibular arch greater than the maxillary arch with the mandibular incisors affected the most. Kassab and Cohen found similar findings in

reviewing cross-sectional epidemiologic studies.<sup>18</sup> They reported that 88% of people 65 years and older had one or more sites with gingival recession. Likewise, 50% of people from the ages of 18 to 64 had one or more sites with gingival recession. Males were more affected than females, and African Americans were the most affected race.

Gingival recession affects the adolescent and young adult population as well. Renkema *et al* evaluated 100 orthodontic patients and 120 control patients in a retrospective case-control study.<sup>19</sup> They followed these patients longitudinally for 8.2 and 9.6 years in the case and control groups, respectively. The authors reported that orthodontic treatment was initiated and completed on subjects at the approximate ages of 12 and 15, respectively. The average length of treatment time was 2.8 years with a range of 1.4 to 4.4 years. At completion of orthodontic treatment, five case patients had 9 sites of gingival recession. Six years post-operatively, 35 case subjects with 105 sites exhibited gingival recession. Twenty age matched controls exhibited 39 sites of gingival recession. The calculated odds ratio of developing gingival recession after completion of orthodontic treatment was 4.48. Thus, the authors concluded that orthodontic treatment promotes the development of gingival recession.

Tooth brushing methods have been implicated by researchers as an etiological factor in the development of gingival recession. According to Sangnes, gingival lesions caused by overly aggressive tooth brushing can be classified into three groups: laceration, gingival retraction, and hyperplasia or hyper-keratinization.<sup>20</sup> Laceration or ulceration lesions are often caused by acute mechanical trauma. In contrast, gingival retraction and hyperplastic lesions are adaptive properties of the gingival tissues to

chronic trauma. Sangnes explains that gingival retraction may be the result of horizontal crossbrushing, and gingival lacerations or clefts may be the result of overzealous vertical brushing. In a study by Khocht et al, subjects who had history of hard toothbrush use and gingival recession were evaluated to determine if a correlation existed.<sup>21</sup> Sixty-three percent of the 182 subjects demonstrated gingival recession. Although the proportion of subjects with recession was approximately the same between men and women, men who had gingival recession were correlated to have a higher percentage of receded surfaces than women. Furthermore, the correlation between percent of receded tooth surfaces and age was statistically significant, as well as the correlation between the percent of receded tooth surfaces and daily brushing frequency. As expected, the subjects who had a positive history of hard toothbrush use demonstrated a significantly higher number of receded tooth surfaces than those with a negative history, 4.5 and 2.3 respectively. Interestingly, the percentage of receded tooth surfaces increased as frequency of tooth brush use increased in only the hard toothbrush users. The group without history of hard brush use did not have an increase in receded surfaces with increased brushing frequency.

In a study by Lang and Löe, the width of facial and lingual keratinized gingiva was evaluated to determine how much keratinized gingiva is needed for the maintenance of gingival health.<sup>22</sup> Thirty-two dental students without periodontal disease underwent a supervised oral hygiene program for 6 weeks, and their teeth surfaces were evaluated for gingival and plaque indices. At the 6 week time point, most surfaces (greater than 80%) with 2.0 mm or more of keratinized gingiva demonstrated gingival health, and 76% of

these same surfaces did not exhibit gingival exudation. On the contrary, all surfaces with less than 2.0 mm of keratinized gingiva demonstrated clinical inflammation and gingival exudate. Lang and Löe concluded that as the width of keratinized gingiva decreased, the gingival index and gingival exudate scores increased. Interestingly, surfaces that were plaque free and had less than 2.0 mm of keratinized tissue still exhibited inflammation. Lang and Löe speculate that the persistence of inflammation on these surfaces is due to a moveable portion of the gingival margin. For example, if a site has less than 2.0 mm of keratinized tissue is actually attached. Unattached moveable tissue could explain the inflammatory state of these teeth surfaces that lack an "adequate amount of keratinized tissue".

Studies have evaluated the need for keratinized tissue in preventing gingival recession. In a study by Kennedy *et al*, 32 patients with an inadequate amount of attached keratinized tissue (< 2 mm) on bilateral sites were treated in a split-mouth design with and without an autogenous free gingival graft at the experimental site.<sup>23</sup> Control sites did not receive any graft and were simply monitored. Both groups were treated with initial scaling, root planning, oral hygiene instruction, and enrolled in a maintenance program. At the 6 year time point, no differences were noted in gingival inflammation, plaque, or increase in gingival recession in both patient groups. However, patients treated with a free gingival graft had significantly greater clinical attachment levels and keratinized tissue width. Interestingly, in a subset of patients that had discontinued participation in the study and its maintenance program, a significant

difference in gingival recession was noted between test and control sites per patient. Control sites were associated with a mean increase of 0.5 mm of gingival recession and further loss of attachment. Test sites that received grafting did not have any additional recession or loss of attachment. The conclusion drawn from Kennedy *et al* was that attached keratinized tissue was not required to maintain periodontal health but in the absence of plaque control, the risk of periodontal breakdown is high. Thus patients that have an inadequate amount of attached keratinized tissue and are unable to maintain proper plaque control are at higher risk of further attachment loss.

Rationale for treatment of gingival recession also includes dentinal hypersensitivity. Leybovich *et al* evaluated the outcome of patients who experience dentinal hypersensitivity after root coverage and restorative procedures.<sup>24</sup> Twenty-six sites were treated in 9 patients in a randomized clinical trial with either coronally advanced flap plus connective tissue graft or Class V composite resin restoration. After accounting for dropouts, 12 sites in the connective tissue graft group and 12 sites in the Class V composite resin group were evaluated. Patients recorded their dentinal hypersensitivity at baseline and at 3 months post operatively using a visual analog scale from 0 to 10. The mean change in dentinal hypersensitivity decreased by 1.25 in the connective tissue graft group and 1.5 the Class V composite resin group. The difference between the two groups did not reach statistical significance. Interestingly, using a visual analog scale from 0 to 10, patients rated the final esthetic outcome of the connective tissue graft at 7.9, which was superior to the score of 5.5 in the Class V composite resin group. The esthetic outcome reached statistical significance between

both groups. Although both connective tissue grafts and Class V composite resin are viable treatment options to treat dentinal hypersensitivity, Leybovich *et al* provides evidence that patients prefer the esthetic outcome of connective tissue grafts.

In a study by Douglas de Oliveira et al, 25 sites of Miller Class I and Class II gingival recession were treated with coronally advanced flap plus connective tissue graft in 22 patients with cervical dentin hypersensitivity, i.e. cold sensitivity.<sup>25</sup> Cervical dentinal hypersensitivity was measured by expressing air via a triple syringe at a distance of 1 cm for 5 seconds, and applying a cold cotton swab to the tooth for 5 seconds. Subjects rated the magnitude of pain they felt on a numerical scale from 0 (no pain) to 10 (extreme pain). Douglas de Oliveira *et al* performed a surgical technique previously described by Bittencourt et al.<sup>26</sup> Two horizontal incisions were made in the interproximal tissue adjacent to the site with gingival recession with the first incision being slightly coronal to the cementoenamel junction and the second being 1 to 2 mm coronal to the first. Intrasulcular incision, full thickness flap reflection, and split thickness periosteal releasing incisions allowed for coronal advancement of the flap. The space between the two horizontal incisions was de-epithelialized, the flap was coronally advanced and both incisions lines would join as one. The exposed root surface was planed, and a connective tissue graft harvested from the palate was secured to the exposed root prior to flap closure. Overall, a statistically significant decrease in cervical dentin hypersensitivity was demonstrated 3 months post-operatively despite only achieving mean defect coverage of 67.9%. Full coverage was achieved in only 11 cases. Interestingly, the amount of defect coverage and increase in keratinized tissue width

correlated moderately with the decrease in cervical dentin hypersensitivity in this study. The authors noted that full recovery from cervical dentin hypersensitivity cannot be accomplished without full root coverage, although improvement can be expected. Douglas de Oliveira *et al* provided evidence that surgical intervention, i.e. root coverage and keratinized tissue augmentation, often significantly reduced pain and increased quality of life in patients who suffer from cervical dentin hypersensitivity.

Nabers was one of the first clinicians to describe the use of free gingival grafts to augment keratinized tissue width.<sup>27</sup> However, it was Sullivan and Adkins who were first to report on the technique that would make this procedure predictable.<sup>28</sup> In their report, Sullivan and Adkins described that previously developed mucogingival surgical techniques had shortcomings in addressing both gingival recession and lack of attached keratinized tissue. They advocated that if "the recession traverses the mucogingival line, the non-keratinized, mobile character of the alveolar mucosa does not lend itself to the maintenance of a healthy marginal complex." Thus, the armamentarium for addressing both gingival recession and lack of attached keratinized tissue needed retooling. For example, an apically positioned flap could correct a patient's lack of attached keratinized tissue but this procedure is only applicable if the patient has a deep vestibule and if the margins of the gingival recession are predominately in keratinized tissue. Likewise, a lateral pedicle flap, which was first described by Grupe and Warren, would only be applicable if an adjacent tooth had copious amounts of attached keratinized tissue.<sup>28, 29</sup> In addition, simply maintaining the gingival recession via curettage and repeated visits failed not only to improve the outcome of the defect but often times failed to prevent the

defect from getting worse.<sup>28</sup> Thus, at the time, the obvious solution was treatment by grafting. The free gingival graft allowed the clinician to "cover part or all of the denuded root while deepening the vestibule and eradicating frenum pull." By grafting these sites, several objectives were addressed with the aim of reestablishing periodontal health, obtaining root coverage, and preventing further attachment loss.

Sullivan and Adkins divided soft tissue defects into four different classes: deep wide, shallow wide, deep narrow, and shallow narrow.<sup>28</sup> Deep wide defects were described as the most difficult type of recession to treat with results often being unpredictable. On the other hand, shallow narrow defects proved to have predictable and superior clinical results than the other defect types. Inferring upon Sullivan and Adkins results, the ability to graft over denuded root surfaces is limited by the size of the avascular root surfaces. This may explain why deep wide defects were unpredictable in achieving the same magnitude of clinical outcome as shallow narrow defects. Miller would add in a later publication that a large vascular recipient bed can help overcome the impediment of avascular root surfaces.<sup>30</sup>

Miller formulated a classification scheme to not only identify the severity of gingival recession but also to calculate whether or not complete root coverage is possible.<sup>31</sup> According to Miller, 100% root coverage is obtained "if the marginal tissue after complete healing is at the cementoenamel junction, *and* the sulcus depth is 2 mm or less, *and* there is no bleeding on probing."<sup>30</sup> Miller demonstrated that complete root coverage can be achieved predictably and in a one-step procedure by using a free gingival graft from the patient's palate and preparing a large vascular recipient bed. In

this study, Miller was using Sullivan and Adkins classification system and found that "deep-wide" recession defects could be treated in a predictable manner. Such an outcome negates the classification system proposed by Sullivan and Adkins in its prognostic ability for root coverage after treatment. Miller would later define a different classification system in order to prognosticate the ability to obtain 100% root coverage after treatment. <sup>28, 30, 31</sup>

The Miller classification system is divided into four different classes of recession. Class I recession is defined as having the marginal gingival tissue receded coronal to the mucogingival junction but not violating this boundary.<sup>31</sup> Furthermore, Class I defects demonstrate no interproximal bone loss, and complete root coverage can be expected after treatment. Class II recession is defined as having the marginal gingival tissue receded to or beyond the mucogingival junction. As with Class I defects, no interproximal bone loss has occurred with Class II defects, and as such, complete root coverage can be expected after treatment. Class III recession is defined as having the marginal gingival tissue receded coronal or apical to the mucogingival junction. Importantly, Class III defects have the presence of interproximal bone loss and or malpositioning of the tooth that prevents accomplishment of complete root coverage after treatment. Only partial root coverage can be expected when treating Class III defects, according to Miller. Lastly, Class IV defects encompass the same criteria as Class III defects with the caveat that Class IV defects have such severe interproximal bone loss and or tooth malpositioning that any root coverage cannot be expected after treatment. Miller's study shows that Class I and Class II treated defects obtained 100%

root coverage in 71 of 79 sites (90%), partial root coverage in 4 of 79 sites (5%), and no root coverage in 4 of 79 sites (5%).<sup>30</sup> The average gain in root coverage was 3.79 mm and probing attachment gain was 4.54 mm.<sup>30</sup>

Interestingly, the key concept of Miller's classification system and its prognostic value to clinicians in determining root coverage after treatment is determined by the interproximal bone height at pretreatment to support a graft. Miller explains that complete root coverage cannot be achieved on teeth that have interproximal bone loss or that have extruded due to the fact that a graft cannot be maintained at the cementoenamel junction of the tooth as this would be "physically impossible".<sup>31</sup> Thus, it is prudent for the clinician to recognize not only the amount of marginal gingival recession but also the amount of interproximal bone loss when assessing patients with acquired mucogingival defects. Furthermore, the clinician should be able to prognosticate, within reason, the amount of root coverage that can be accomplished before treatment is rendered so that the patient is fully aware of expectations and outcomes of undergoing root coverage procedures. Finally, the clinician can extrapolate long term treatment outcomes based upon primary root coverage and secondary root coverage. Primary root coverage is identified immediately after the grafting procedure, i.e. where the graft is positioned after being secured, where secondary root coverage describes the amount of "creeping attachment" that can occur during the healing process.<sup>30</sup> Thus, the final amount of root coverage may not always be easily predicted considering that an average of 1.2 mm of creeping attachment may occur one year post operatively.<sup>32</sup>

Miller's report included key concepts that are still used today in gingival graft procedures. Root preparation with a curette to remove root convexity and concavity along with root conditioning allows for connective tissue attachment between the graft and the denuded root surface.<sup>30</sup> Creation of butt-margins at the recipient site at the level of the cementoenamel junction, just below the papilla, aids the clinician in later suturing and securing the graft. In addition, securing the graft so that mobility is minimized is accomplished with the use of interrupted sutures at the coronal portion of the graft through the adjacent papilla and interrupted sutures at the apical base of the graft into periosteum. Finally, placement of a periodontal dressing can be used but healing appears to show no difference in mucogingival surgical procedures with or without its use.<sup>33</sup>

In addition to his classification system, Miller discussed factors that were commonly associated with incomplete root coverage. Miller advocated the following to avoid complications: 1) classify the gingival recession defect according to Miller's classification scheme; 2) remove any root anatomic features that would hinder healing by "flattening" the root surface; 3) condition to root surface with citric acid to remove the "smear layer" and open dentinal tubules to allow for connective tissue and long junctional epithelial attachment; 4) proper placement of butt-joint margined incisions with horizontal incisions at the cementoenamel junction and vertical incisions at the line angles of adjacent teeth; 5) graft must be of adequate size and thickness and when in doubt err on a larger , thicker graft; 6) avoid pressure, trauma, or the introduction of smoking during the post-operative healing period.<sup>34</sup>

Typically, the clinical outcome following root coverage surgery is measured by the percentage of root that is left exposed. For example, a tooth with 6 mm of gingival recession is treated and now has 2 mm of residual gingival recession. This tooth is said to have 66% root defect coverage since only 66% of the original defect is covered by gingiva after treatment. Greenwell et al explains that reporting of outcome in this manner is deceptive.<sup>35</sup> Sixty-six percent is a less than optimal figure in most clinical outcomes. Instead, Greenwell et al recommends describing the baseline and treated measurements as a percentage of the root length, i.e. total root coverage versus root defect coverage. For example, a tooth with 6 mm of gingival recession also has a root length of 12 mm. Its baseline percentage of root coverage is 50% since 6 mm of gingiva is covering 12 mm of root length. After treatment, the tooth now shows 2 mm of gingival recession. Its post-treatment percentage of root coverage is now calculated as 83.3% since 10 mm of the gingiva is covering 12 mm of root length. As previously mentioned, the current reporting practice would state that the treatment resulted in 66% of root coverage when in fact 83.3% of the total root is actually covered. Since the actual root length of every tooth that is treated is often unknown, Greenwell et al recommends a generally accepted universal root length of 13.63 mm. Craft et al demonstrated that single mean root length of all roots in the mouth (excluding palatal roots) is 13.63 mm.<sup>36</sup> With the reporting practice advocated by Greenwell *et al*, a direct comparison can be made in percentages between baseline and post-treatment root coverage values. Furthermore, Greenwell et al define success criteria for defect coverage and root coverage. Successful mean defect coverage is achieved when 80 to

100% of the defect is covered at least 75% of the time. Successful defect elimination is achieved when 95 to 100% mean root coverage is obtained, and 90% coverage is obtained 90% of the time.

Free gingival grafts heal with an esthetic disparity in comparison to other mucogingival techniques. Often times the grafted gingival tissue does not blend well with adjacent tissues and the result can be described as a "keloid" or "tire-patch" formation. Furthermore, the free gingival graft procedure requires that donor tissue be taken from the patient's palate thus leading to increased post-operative morbidity. Other techniques have been developed to address the limitations with free gingival grafts. The "coronally repositioned periodontal flap", or the "coronally advanced flap" as it is often termed today, was first reported by Bernimoulin *et al* as a two stage procedure to treat gingival recession.<sup>37</sup> First the keratinized tissue apical to the recession site was augmented with a free gingival graft harvested from the palate. After adequate healing of 2 months, a second procedure was performed in which a trapezoidal flap was created and subsequently coronally positioned after periosteal releasing incisions were made. Bernimoulin et al gives credit to Harvey et al as the first to report the use of the following technique. <sup>37, 38</sup> Specifically, the incision design calls for intrasulcular incisions with two vertical incisions made at the line angle adjacent to the teeth with gingival recession. Next, a new papilla tip is created by forming a v-shaped incision at the base of the existing papilla, with the "v" pointed coronally. Afterwards, the epithelium coronal to the new papilla tip is removed with ophthalmic scissors. A full thickness flap was then elevated and periosteal incisions were created at the base of the

flap to allow for coronal advancement. Sutures were placed at the lateral borders of the flap and then followed by sutures at the papillae. No periodontal dressing was used.

The coronally advanced flap was further discussed by Allen and Miller.<sup>39</sup> In their study, Allen and Miller described the coronally advanced flap as a single stage procedure without previous or simultaneous grafting on specific gingival recession defects. In Allen and Miller's study, 37 sites of gingival recession in 28 patients were treated. These were Class I recession defects that had a minimum of 3 mm width and 1 mm thickness of keratinized tissue present at the apical extent of the defect. The root surface underwent curettage with hand instruments and later conditioned with citric acid. Two vertical incisions were made lateral to the defect and were extended from the apical portion of the papilla to the alveolar mucosa as to create a trapezoidal flap. A sulcular incision was then followed by full thickness flap elevation to the mucogingival junction. Split thickness dissection beyond the mucogingival junction allowed for coronal positioning of the flap. Gingivoplasty was then performed to remove the epithelium at each papilla to allow for the connective tissue beds of the recipient site and flap to be in intimate contact. The vertical incisions were sutured prior to suturing the papillae. The baseline amount of recession of the 37 sites averaged 3.25 mm, and the procedure improved this value to 0.07 mm at 6 months post-operatively. Thus, the mean gain of root coverage was 3.18 at 6 months post- operatively. Overall, 84% of sites treated had complete root coverage. These results are comparable to Miller's outcome values with free gingival grafts with the caveat that the patients treated with the coronally advanced flap had adequate keratinized tissue prior to treatment.

Treatment of multiple gingival recession defects using the coronally advanced flap in patients with esthetic demands was investigated by Zucchelli and De Sanctis.<sup>40</sup> Their rationale for treatment of multiple gingival recession defects simultaneously is to minimize patient discomfort by decreasing the number of surgical sites and appointments, and to improve esthetic outcomes by eliminating graft placement at the recession defect. The authors state that often times a palatal tissue graft may cause excessive thickness and poor contour and color blending of the treated area. Twentytwo healthy patients who presented with at least two gingival recession defects adjacent to the esthetic zone were enrolled in this case series. All defects were Miller Class I and II defects and did not suffer any interproximal soft or hard tissue loss. Surgery began with oblique submarginal incisions in the interdental area and intrasulcular incisions at the recession defects. A surgical papilla was created with the interdental oblique incisions so that the "surgical papilla mesial to the flap midline were dislocated more apically and distally, while the papilla distal to the mid-line were shifted in a more apical and mesial position." The incision design extended one tooth on each side of the teeth to be treated as to allow for coronal repositioning of the flap. The envelope flap was elevated in a "split-full-split" fashion in the coronal to apical direction, i.e. splitthickness dissection at the surgical papilla, full-thickness elevation of the gingival tissues apical to the root exposure, and split-thickness dissection beyond the mucogingival junction to allow for passive coronal displacement of the flap to the cementoenamel junction. The facial aspects of the remaining anatomic papillae were de-epithelialized to allow for a connective tissue to connective tissue interface between both the surgical and

anatomic papillae. The papillae were sutured with interrupted sling sutures while the base of the flap was secured with a horizontal mattress suture as to eliminate tension from muscle pull. The mean number of gingival recessions treated in each subject was 3.4, and the mean recession depth was 2.8 mm. The mean recession depth decreased to 0.1 mm at one year following the procedure. Interestingly, the study demonstrated an increase in keratinized tissue width with the coronally advanced flap even though no grafts were used. This may be explained by genetic factors that cause the mucogingival junction to reposition itself in accordance to adjacent untreated sites <sup>41</sup>. However, all treated sites had an existing band of keratinized tissue and the above results cannot be extrapolated to include gingival recession defects that lack keratinized tissue.

Another variant of the coronally advanced flap is the semilunar coronally repositioned flap as described by Tarnow.<sup>42</sup> The semilunar coronally repositioned flap is indicated in patients who have an adequate band of keratinized tissue, high esthetic concerns, and minimal facial probing depths. After scaling and root planning the exposed root, a semilunar incision is made paralleling the facial gingival margin of the tooth showing recession. The incision can be carried beyond the mucogingival junction if needed depending upon the keratinized tissue width at the site. The incision should terminate at the base of the adjacent papilla leaving 2 mm of unaltered gingiva coronally for blood supply. Ideally, the apical extent of the flap should reside on bone for additional blood supply. Split thickness dissection with a 15C blade allows for coronal repositioning of the semilunar flap. Firm pressure is then applied to the flap for 5 minutes with moist gauze. No sutures are placed at the surgical site although a dressing

is used. In 20 teeth treated by Tarnow using this method, none resulted in sloughed flaps. Approximately 2 to 3 mm of root coverage could be obtained with the semilunar flap technique.

In a study by Baldi *et al*, 19 Miller Class I and II recession defects with 2 mm or greater vertical recession were treated by coronally advanced flaps in order to determine if flap thickness had any impact on root coverage.<sup>43</sup> After scaling and root planing the site of root exposure, an intrasulcular incision was made and carried out horizontally into the interproximal area. The horizontal incisions stopped short of the adjacent tooth cementoenamel junction. Two vertical releasing incisions were carried out at the termination of the horizontal incisions to create a trapezoidal envelope flap. Full thickness flap elevation followed by partial-thickness dissection allowed for coronal advancement of the flap. The flap thickness was measured at the mid-point between the mucogingival junction and the gingival margin with a modified Iwansson gauge. At baseline, mean vertical recession was 3.0 mm, and the mean flap thickness was 0.7 mm. Three months post-operatively, mean vertical recession decreased to 0.6mm. Multiple linear regression analysis demonstrated a statistically significant association between recession reduction and both flap thickness and vertical recession depth. All treated sites that had a flap thickness of more than 0.8 mm demonstrated complete root coverage. Sites that had a flap thickness of 0.8 mm or less showed only partial root coverage.

Pini Prato *et al* evaluated flap tension and its effects on root coverage in 11 patients who were treated for gingival recession via coronally advanced flap technique.<sup>44</sup> Each patient had two contralateral sites with 2 mm or more of gingival recession. After

receiving a trapezoidal envelope flap, each side was randomly selected to receive full thickness flap elevation plus partial thickness dissection or full thickness flap elevation alone. Partial thickness dissection was deemed complete when the flap could be coronally advanced tension free. Tension was verified with the use of a dynamometer. Each flap was positioned to the cementoenamel junction and secured with 5-0 silk suture. There was no statistically significant difference in the positioning of the flap to the cementoenamel junction in either group. The mean vertical recession depth at baseline was 2.75 mm for all 22 sites. Three months post-operatively, gingival recession decreased by a mean of 2.25 mm. The test sites that did not receive partial thickness dissection had a mean flap tension of 6.5 g at the time of surgery. Tests sites had a mean recession depth of 2.82 mm at baseline, and 3 months post-operatively recession depth improved to 0.64 mm. Control sites that did receive partial thickness dissection had a mean flap tension of 0.4 g. Control sites had a mean recession depth of 2.68 mm at baseline, and 3 months post-operatively recession depth improved to 0.36 mm. Mean gingival recession reduction was 2.18 mm and 2.32 mm in the test and control sites, respectively. Interestingly, the difference in gingival recession reduction was not statistically significant between the test and control groups. According to Pini Prato et al, flap tension at time of surgery does not impact gingival recession reduction at three months post-operatively. The final gingival margin position at the time of surgery appears to be of more importance than flap tension, or lack thereof.

Likewise with free gingival grafts, coronally advanced flaps presented with shortcomings in the treatment of gingival recession. Although no procedure is without

morbidity post-operatively, the use of connective tissue graft with flap advancement is currently accepted as the standard of care. In fact, connective tissue grafts are still considered the "gold standard" in treatment of gingival recession.

Raetzke was the first to describe utilizing autogenous connective tissue grafts in the treatment of gingival recession.<sup>45</sup> Ten patients with 12 areas of recession were treated. At each recession site, a small collar of tissue with the sulcular epithelium was removed followed by thorough scaling and root planning. The exposed root was then conditioned with citric acid in the same manner described by Miller.<sup>30, 45</sup> A partial thickness flap or "envelope" was created in the tissue adjacent to the denuded root surface. Raetzke harvested connective tissue at the premolar/molar region on the palate beginning with two incisions that were 1 to 2 mm apart and were made in the anterior/ posterior direction. The length of the graft was double the width of the gingival defect. The graft was then carefully removed and placed underneath the "envelope". The epithelium on the graft can optionally be removed or left intact. Post-operative measurements were recorded 2 to 8 months after surgery. The average root coverage obtained was 80% with 42% of sites obtaining full root coverage. Keratinized tissue width increased on average by 3.5 mm. Furthermore, Raetzke stated that the advantage of his "envelope" technique arises from minimal surgical trauma. The donor site healed by primary intention since the wound margins can be approximated and sutured closed. The recipient site was undermined via partial thickness only, and the preparation bed was not left exposed. In addition, Raetzke elaborated that the esthetic appearance of the connective tissue graft matches the surrounding tissue and does not have a "patchy"

appearance. The shortcoming of this procedure, however, lies in the amount of connective tissue that can be harvested from the patient's palate, which becomes especially disconcerting when attempting to address multiples sites of gingival recession.

Langer and Langer modified Raetzke's "envelope" technique to allow for treatment of multiple sites of recession.<sup>46</sup> Specifically, two vertical incisions are "placed at least one-half to one tooth wider mesiodistally than the area of gingival recession." Horizontal incisions are placed at the base of the papilla as to leave the interproximal tissue intact for later suturing. A partial thickness flap was created carefully with sharp dissection at the recipient site, and the exposed root surface was scaled. The donor site was prepared in the palate by first placing a horizontal incision 2 to 3 mm apical to the free gingival margin of the adjacent teeth. A second incision was created 1.5 to 2 mm apart from the first one. The length of the horizontal incisions was determined by the length of the number of teeth involved at the recipient site. Two vertical incisions are then placed to facilitate flap elevation and connective tissue graft removal. The donor site was immediately sutured after graft procurement. The graft was then placed at the recipient site with both connective tissue and epithelial collar intact. The graft and flap were secured with suture, and no attempt was made to coronally advance the flap with Langer and Langer's approach. The authors report an increase in root coverage anywhere from 2 to 6 mm.

The double papillae repositioned flap was first described by Cohen and Ross.<sup>47</sup> With this approach, the gingival recession could be treated by two laterally placed pedicle flaps donated from the mesial and distal margins of the recession site.

Specifically, two horizontal incisions were created at the base of the interproximal papillae on either side of the tooth with the recession defect. Two vertical incisions were then made from the horizontal incision at the line angle of the adjacent teeth. A small cut-back incision may be needed to facilitate laterally placing each pedicle onto the denuded root surface. Sutures secured each pedicle to each other and finally to the wound margins. Nelson modified this approach by introducing a connective tissue graft to the recipient site which was covered by the two laterally positioned pedicle flaps.<sup>48</sup> Nelson's results were comparable with previous studies thus covered. Patients treated with advanced recession, which Nelson defines as 7 to 10 mm of recession, had an average of 88% root coverage with half of this group demonstrating complete root coverage. Results were more favorable in treatment of mild and moderate recession sites. Nelson did not identify if there was any interproximal bone or soft tissue loss at baseline of any defect treated.

The evidence available to researchers led many to ask the question whether or not a graft was necessary in the treatment of gingival recession. The work of Pini Prato *et al* attempted to answer such a question with a split-mouth study design and 5-year follow up data.<sup>49</sup> Thirteen patients with multiple sites of gingival recession bilaterally were treated with coronally advanced flap or coronally advanced flap plus connective tissue graft. A total of 93 Miller Class I, II, and III gingival recession sites were treated. At the 6-month time point, there was no difference in the amount of complete root coverage between both groups. However, at the 5-year time point, the group treated with a connective tissue graft exhibited a greater percentage of sites with complete root

coverage (52% versus 35%). Furthermore, an apical relapse of the gingival margin could be seen in the group treated without the graft. On the contrary, sites treated with a graft showed coronal displacement of the gingival tissue. Inferring from Pini Prato *et al*, grafts appeared to be essential for maintaining long term results in the treatment of gingival recession.

Zucchelli *et al* provided additional evidence to answer whether or not a graft was needed in the treatment of gingival recession defects.<sup>50</sup> The coronally advanced flap was compared with and without a connective tissue graft in the treatment of multiple adjacent gingival recession defects in the esthetic zone. Fifty subjects were divided equally between the two treatment groups in this double-blinded, randomized controlled clinical trial. Subjects were treated in a manner similar to the manner described before by Zucchelli and De Sanctis with the caveat that the test group patients were treated with connective tissue grafts.<sup>40, 50</sup> The study demonstrated that there were no statistically significant differences in gingival recession reduction and complete root coverage between both groups at the 6 month and 1 year time point. However, after 5 years, the group treated with a coronally advanced flap and connective tissue graft displayed significantly greater recession reduction, greater probability of complete root coverage, greater buccal keratinized tissue height, and better soft tissue contour than the group treated with coronally advanced flap alone. Conversely, the group treated with the coronally advanced flap alone had better color match and a better post-operative course than the group treated with connective tissue graft. Thus, Zucchelli *et al* provides evidence that a graft is recommended to maintain root coverage stability long-term.

Although the techniques mentioned thus far showed favorable results with connective tissue grafts and free gingival grafts, procurement of the graft from the palate has certain anatomic considerations and limitations. Care must be taken to not damage the greater palatine neurovascular bundle during graft harvesting as this could complicate achieving hemostasis. Typically, the greater palatine neurovascular bundle arises at the junction of the vertical and horizontal sections of the palatine bone, apical to the third molar. This bundle then courses anteriorly within a bony groove. Reiser *et al* studied human cadavers and created a classification system to define palatal vault height.<sup>51</sup> Specifically, the distance from cementoenamel junction to the neurovascular bundle was 7 mm, 12 mm, and 17 mm in shallow, average, and high palatal vaults, respectively. The distance was taken in the area of the maxillary molars and premolars. Furthermore, this cadaver study revealed that tissue is thickest in the palate between the mesial line angle of the palatal root of the first molar to the distal line angle of the canine. The findings in Reiser et al define the anatomic limitations of harvesting from the palate.

Complications in donor site healing, availability of donor tissue, and postoperative discomfort led some researchers to investigate an alternative graft source. The hallmark study by Harris led to the current era of soft tissue allografts for the treatment of gingival recession.<sup>52</sup> Harris treated 50 patients with 107 sites of gingival recession to determine if a soft tissue allograft material was as effective in root coverage as autogenous connective tissue grafts. The study population was divided into two treatment groups of 25 patients. The control group consisted of 42 recession defects that

were treated with a coronally advanced flap combined with a connective tissue graft. The 65 recession defects that made up the test group were treated in the same manner, except that an acellular dermal matrix (AlloDerm<sup>®</sup>) substituted the use of a connective tissue graft. As a prerequisite, all patients had to have 2 mm of gingival recession or more, and the interproximal tissue of the treated teeth must be coronal to the cementoenamel junction. Thus, Miller Class I, II, and III defects were included in the study. The exposed root surfaces were planed by hand, ultrasonic, and rotary instruments, and were conditioned with a 125mg tetracycline/ mL saline solution for 3 minutes. Horizontal incisions were created laterally from the cementoenamel junction of the tooth with the recession and carried over to the adjoining teeth mesially and distally. Sulcular incisions were then made to connect the horizontal incisions. Care was taken to avoid any vertical incisions. Periosteal releasing incisions were accomplished by sharp dissection until the flap could be advanced coronally without tension. Gingivoplasty at the papilla adjacent to the treated teeth created a bleeding bed. In the control group, a connective tissue graft was harvested from the palate via a double horizontal incision technique and secured to the recipient bed with resorbable suture. In the test group, the acellular dermal matrix was trimmed so that it extended 3 mm over the adjacent bone, and then sutured with resorbable suture. Lastly, the buccal flaps in both groups were secured with chromic gut suture. Both groups showed no statistically significant differences at baseline in regards to clinical measurements. At 12 weeks postoperatively, both groups showed significant improvement in root coverage with a mean of 96.2% and 95.8% in the control and test groups, respectively. The difference in root

coverage between the groups was not statistically significant. Complete root coverage was obtained in 81.0% and 87.7% of control and tests groups, respectively. The mean increase in keratinized tissue width was 2.0 mm and 1.2 mm in the control and test groups, respectively. The mean probing depth change in the control and test groups were 1.2 mm and 0.7 mm, respectively. The difference in keratinized tissue width and probing depth change was statistically significant, in favor of the control group. Although the acellular dermal matrix did not perform as well as the connective tissue graft in keratinized tissue augmentation and probing depth reduction, it does appear to be as effective as connective tissue grafts in root coverage. Importantly, the Harris study supports the use of soft tissue allografts in not only the treatment of gingival recession, but in the treatment of multiple sites of gingival recession that would otherwise be discouraged with connective tissue grafts.

Cummings *et al* assessed the wound healing process of connective tissue grafts and acellular dermal matrices through histology in 12 human teeth.<sup>53</sup> All four patients who participated in this study were smokers, had gingival recession defects that ranged from 3 to 6 mm, and had an anterior tooth that was treatment planned for extraction. Six months prior to extraction, each study tooth was planed with hand instruments and conditioned with 24% ethylenediaminetetraacetic acid (EDTA). Each tooth then received intrasulcular incisions connected to horizontal incisions in the interproximal area followed by vertical incisions in the mesial and distal extent of the flap design. A full thickness flap was elevated followed by split-thickness facial flap sharp dissection to facilitate coronal advancement of the flap. Prior to closure, each tooth received either a

connective tissue graft, an acellular dermal matrix, or no graft. After six months healing, the study teeth were extracted en-block. The teeth that did not receive a graft served as control teeth. Sulcular epithelium and junctional epithelium attachment were apparent on the control roots with a mean width of 0.62 mm and 1.6 mm, respectively. At the root surface, collagen fibers were seen in parallel arrangement with the root and had a mean of 0.54 mm of attachment. The teeth that received connective tissue grafts had sulcular epithelium and junctional epithelium attachment of 0.57 mm and 0.97 mm on the treated roots, respectively. There was a similar amount of attachment between the connective tissue and control groups. Although the organization of the connective tissue in the area coronal to the osseous crest was somewhat disorganized, the collagen fibers in this area were generally parallel with the root surface. The connective tissue attachment had a mean width of 1.04 mm, and had an increased buccolingual thickness as compared with control teeth. No elastin fibers were noted in the dense collagen of the overlying gingiva or in the graft. The teeth that received acellular dermal matrix grafts had sulcular epithelium and junctional epithelium attachment of 0.47 mm and 1.17 mm on the treated roots, respectively. These measurements were similar to both control and connective tissue grafted teeth. The grafted material was visually similar in composition in standard hematoxylin and eosin stain. The connective tissue attachment had a mean width of 1.13 mm, and like the connective tissue graft group, the acellular dermal matrix group demonstrated an increased buccolingual thickness when compared to the control teeth. Overall the collagen fibers were parallel to the root surface. Interestingly, elastin fibers were noted in abundance in the acellular dermal matrix graft when the specimen

was stained with Verhoeff's solution. As such, the graft can be readily distinguished from native connective tissue and mucosa with the Verhoeff's solution. In conclusion, the tissue overlying treated tooth roots are similar histologically but with one key difference: acellular dermal matrix has elastic fibers while the connective tissue grafts do not.

Soft tissue allografts have grown in popularity as a treatment alternative to autogenous tissue. However, some patients may feel more comfortable in accepting donor material from an animal source rather than a human source.

In a case report, Rotundo and Pini- Prato described the use of a "new Collagen Matrix" in the treatment of multiple gingival recessions in 3 non-smoking women.<sup>54</sup> Eleven maxillary teeth with gingival recession were treated with a coronally advanced flap plus a porcine derived, two-layer xenogenic collagen matrix (Mucograft<sup>®</sup>, Geistlich). Specifically, "a linear intrasulcular/ interdental partial-thickness flap was performed". Then, "oblique interdental incisions were inverted in correspondence with the middle axis of the flap" so that new surgical papillae were created. Full-thickness flap elevation was carried out to the mucogingival junction, and transitioned to split-thickness flap by sharp dissection of the periosteum and inner muscle insertions. Exposed root surfaces were scaled and root planed. The collagen matrix was trimmed and secured over the defect area via 6-0 resorbable sutures. The flap was then coronally positioned and secured with sling sutures. In one patient, a tunnel technique was used between the two maxillary central incisors that were being treated along with a distal vertical releasing incision to allow for coronal advancement of the flap. The 11 treated

defects showed a gingival recession mean of 2.9 mm, keratinized tissue width mean of 2.45 mm, and a probing depth mean of 1.73 mm at baseline. After 1 year, 9 of 11 treated sites demonstrated complete root coverage, and the mean keratinized tissue width increased to 3.1 mm. Rotundo and Pini- Prato' case study provides evidence of the potential of using a xenogenic collagen matrix in the treatment of gingival recession, but stronger evidence would be needed to substantiate the new material.

Cardaropoli *et al* completed a randomized controlled clinical trial in 32 patients using coronally advanced flap with or without a xenogenic collagen matrix (Mucograft<sup>®</sup>, Geistlich).<sup>55</sup> A total of 113 Miller Class I and II gingival recession defects were scaled and planed, and received a flap design that was previously described by Zucchelli and De Sanctis.<sup>40, 55</sup> The xenogenic collagen matrix was trimmed and positioned to the cementoenamel junction and secured with 5-0 resorbable sutures in the 58 test sites while no graft was used in the control sites. The flap in both groups received partial thickness dissection so that the flap could be positioned to the cementoenamel junction, tension free. Flaps were secured with 5-0 non-resorbable suture. At baseline, the mean gingival recession depth was 3.63 mm and 3.38 mm in the test and control groups, respectively. There was no statistically significant difference in gingival recession depth between the two groups at baseline. Twelve months post-operatively, the mean gingival recession depth was 0.20 mm and 0.58 mm in the test and control groups, respectively. The difference in gingival recession reduction between the two groups was statistically significant, favoring the test group. Furthermore, complete root coverage was achieved in 72% of the tests sites and 58% of the control sites, and this difference was statistically

significant. According to Cardaropoli *et al* treatment of gingival recession with a coronally advanced flap plus xenogenic collagen matrix was more effective than a coronally advanced flap alone.

A porcine derived extracellular matrix (DynaMatrix<sup>®</sup>, Keystone Dental) was investigated by Nevins *et al* as a potential alternative to autogenous free gingival grafts in the treatment of keratinized tissue augmentation.<sup>56</sup> DynaMatrix is acquired from the submucosal tissues of the small intestine in pigs, and is processed in such a way that retains the natural composition of matrix molecules like collagen types I, III, IV, and VI, glycosaminoglycans, glycoproteins, proteoglycans, and growth factors <sup>57</sup>. Six patients who presented with less than 2 mm of attached keratinized gingiva bilaterally on the facial aspect of the mandibular posterior teeth were treated via a randomized, controlled split-mouth study design. The test sites received the porcine derived extracellular matrix while the control sites received an autogenous free gingival graft. Both sides in the same patient were treated at the same appointment. Briefly, a horizontal incision was made with a number 15 blade at the mucogingival junction of the defect area. A partialthickness flap was dissected to create a recipient bed for either graft material. The graft material was secured via polytetrafluoroethylene sutures and a periodontal dressing. After 13 weeks of healing, both the test and control sites demonstrated no statistically significant differences in probing depth, vertical gingival recession, horizontal gingival recession, and keratinized tissue width between each group. Furthermore, a biopsy punch was taken in five pairs of test and control sites for histologic study. Both groups were determined to be similar histologically. Specifically, both groups demonstrated

"mature connective tissue covered by keratinized epithelium", having a "small band of dense orthokeratinization at the top of the epithelium", and having rete pegs in similar size and appearance. In addition, a few subjective observations were noted by the authors of this study. First, the esthetic outcome of the test group appeared to be superior to the control group since tissues were better matched in color and in shape. Next, patients reported less discomfort with the test sites, mainly due to the lack of donor site post-operative pain. Last, the authors hypothesize that "the epithelium that populated the DynaMatrix membrane migrated from the denuded epithelium by 'creeping over' the wound bed. This is probably a result of the unique scaffold that allowed repopulation of fibroblasts, blood vessels, and epithelium from the surrounding tissues". Thus, Nevins' study gives evidence that a xenograft can deliver the same clinical and histological outcomes as an autogenous graft in augmenting keratinized tissue.

Porcine derived extracellular matrix is processed from the submucosal tissues of the small intestine in pigs in such a way that retains the natural composition of matrix molecules like collagen types I, III, IV, and VI, glycosaminoglycans, glycoproteins, proteoglycans, and growth factors. In a study by Hodde *et al*, the effects of disinfection and sterilization on the growth factors, glycoproteins, glycosaminoglycans, and three dimensional matrix structure of porcine small intestinal submucosa were examined.<sup>57</sup> Porcine derived extracellular matrix obtained from the jejunum is processed with an oxidizing agent, followed by lyophilization, and then sterilized with ethylene oxide gas. Specifically, the porcine jejunum is acquired immediately after slaughter, rinsed with

water, and split longitudinally to form a sheet. Unneeded portions of the harvested tissues, i.e. the tunica muscularis externa, the tunica serosa, and the superficial layers of the tunica mucosa, are removed by mechanical delamination. The resultant porcine small intestine submucosa is then treated with a diluted solution of peracetic acid for 2 hours, and then subjected to freezing and lyophilization in order to produce a dry sheet. The disinfected porcine small intestine submucosa was then packaged into gas permeable pouches to allow for sterilization with ethylene oxide. Hodde et al investigated different intervals in the above tissue processing sequence in order to observe differences in TGF $\beta$ 1, VEGF, and FGF-2. Immediately after jejunum procurement and mechanical delamination, the porcine small intestinal submucosa TGF $\beta$ 1 and VEGF values were 4,841 pg/g and 26,655 pg/g, respectively. After the tissue was subjected to paracetic acid, TGF $\beta$ 1 and VEGF values dropped to 892 pg/g and 159 pg/g, respectively. Ethylene gas sterilization further reduced TGF $\beta$ 1 and VEGF values to 711 pg/g and 130 pg/g, respectively. FGF-2 demonstrated a value of 49,902 pg/g after porcine jejunum procurement and mechanical delamination, but showed a stark increase after disinfection with paracetic acid to 105,537 pg/g. The increase can be explained by the "removal of the cellular component mass" thus allowing more FGF-2 to be detected. However, FGF-2 decreased to 26,736 pg/g following ethylene gas sterilization. Growth factors TGF $\beta$ 1, VEGF, and FGF-2 were susceptible to the disinfection and sterilization process yet a significant portion of the growth factors still remained. Most of the glycoprotein, fibronectin, was preserved during the disinfection and sterilization processing of the small intestine submucosa showing no significant

decrease before and after treatment (686 ng/ g to 425 ng/ g). Detectable sulfated glycosaminoglycans actually increased through the disinfection and sterilization process, achieving values of  $3.34 \,\mu$ g/mg and  $10.20 \,\mu$ g/mg before and after disinfection/ sterilization, respectively. Hyaluronic acid too is highly retained after tissue processing achieving values of  $1990 \,\mu$ g/g and  $1872 \,\mu$ g/mg before and after disinfection/ sterilization, respectively. Electron microscopy revealed that after disinfection and sterilization, the porcine small intestinal submucosa "lost its cellular and lipid components, resulting in a more homogenous, fibrous scaffold". By removing non-collagenous constituents, voids are created that can facilitate host cell migration. Furthermore, the perpendicular fiber orientation of the native tissue was maintained. Overall, the "material maintains the three-dimensional architecture and topographical features of the original small intestinal submucosa tissue".

In another study by Hodde *et al*, the ability of porcine small intestinal submucosa to support fibroblast attachment and induce proliferation was evaluated.<sup>58</sup> Porcine small intestinal submucosa was disinfected and processed in the same manner that was previously reported by Hodde *et al*.<sup>57, 58</sup> Small intestinal submucosa was disinfected and sterilized through the following steps: peracetic acid, lyophilization, and ethylene oxide gas. An alamarBlue assay was used to evaluate attachment and viability of seeded fibroblasts on the small intestinal submucosa following various stages of the above mentioned disinfection and sterilization process. For the sample of tissue treated with peracetic acid, 66 % of seeded fibroblasts attached to the tissue during the 1-hour incubation period and remained viable for an additional 18 hours. Following

lyophilization and ethylene oxide gas sterilization, 57% and 51% attachment was observed, respectively. No statistically significant differences were detected in the percentage of attachment of viable fibroblasts in each of the 3 groups. Furthermore, the effects of the disinfected and sterilized submucosal tissue was evaluated for its stimulatory effects of murine pheochromocytoma (PC12) cells, and for its ability to upregulate VEGF secretion by attached fibroblasts. When PC12 cells were subjected to certain growth factors, i.e. laminin, NGF, and FGF-2, they produce neurite-like extensions. Twenty-one percent of PC12 cells that were attached to disinfected and sterilized small intestinal submucosa were able to produce neurite-like extensions. In addition, fibroblast cell cultures that contained the disinfected and sterilized small intestinal submucosa produced significantly greater levels of VEGF at the 16 and 24 hour time points than controls. Thus, Hodde *et al* provided evidence that a disinfected and sterilized tissue retained growth factors that aid in fibroblast attachment and induced up-regulation of fibroblasts and other cell types.

To the author's knowledge, there are no randomized clinical trials that investigate the use of DynaMatrix Plus<sup>®</sup> in the treatment of gingival recession. DynaMatrix Plus<sup>®</sup> is a double layered version of DynaMatrix<sup>®</sup> made specifically for the treatment of gingival recession defects. The product is indicated to be used in conjunction with a coronally advanced flap. The purpose of this study is to compare the percentage of root coverage and clinical attachment levels in areas of localized marginal tissue recession between DynaMatrix Plus<sup>®</sup> and autogenous connective tissue graft, the current gold standard, in a randomized clinical trial.

#### CHAPTER II

# A COMPARATIVE STUDY OF ROOT COVERAGE USING DYNAMATRIX PLUS VERSUS CONNECTIVE TISSUE GRAFT

#### **II.1 Introduction**

Gingival recession affects a wide variety of people of all ages. A 2003 study found that more than 50% of people ages 18 to 64 and 88% of people over the age of 65 had one or more sites of gingival recession.<sup>18</sup> Etiological factors for gingival recession include orthodontic treatment, overly aggressive tooth brushing, use of a hard bristle toothbrush, and the combination of poor oral hygiene with a lack of attached keratinized tissue.<sup>19-23</sup> Indications for gingival recession treatment include dentinal hypersensitivity, esthetic demands, prevention of further gingival recession, and class V carious lesions.<sup>24,</sup> 25, 31

Free gingival grafts, coronally advanced flap (CAF), semi-lunar flap, and combination of CAF plus autogenous connective tissue graft (CTG), allograft, or xenograft are some of the techniques reported in the literature to treat gingival recession.<sup>30, 37, 38, 40, 42, 45, 46, 52</sup> Today, the gold standard in treatment of gingival recession remains CAF plus CTG. Pini Prato *et al* and Zucchelli *et al* evaluated the clinical outcome of CAF versus CAF plus CTG to determine if grafts were necessary for root coverage.<sup>49, 50</sup> Although short term outcomes (<6 months) between groups showed no difference, grafts appeared to be essential for maintaining long term ( $\geq$ 5 years) results in the treatment of gingival recession. CAF plus CTG requires harvest of the underlying

connective tissue from the palate and its placement at the cementoenamel junction (CEJ) of the tooth with the recession defect.

CAF plus CTG requires a second site of surgical morbidity often leading to greater patient discomfort and increased surgical time.<sup>52</sup> Complications in donor site healing, availability of donor tissue, and post-operative discomfort led some researchers to investigate an alternative graft source. Acellular dermal matrix (ADM) is a connective tissue matrix allograft that acts as a scaffold for fibroblasts to migrate into and is compatible for epithelial cell migration from adjacent tissue margins.<sup>53</sup> Processing of ADM involves removing the epidermal layer along with its cellular structures so that factors responsible for graft rejection and infection are limited.<sup>59</sup> Harris demonstrated that ADM is a viable substitute for CTG in the treatment of gingival recession defects.<sup>52</sup> Harris reported mean percent defect coverage of 96.2% and 95.8% in CTG and ADM groups, respectively, and the difference was not statistically significant. Moreover, Cummings *et al* showed that wound healing of ADM and CTG are histologically similar.<sup>53</sup>

Soft tissue allografts have grown in popularity as a treatment alternative to autogenous tissue. However, some patients may feel more comfortable in accepting donor material from an animal source rather than a human source. In a case report, Rotundo and Pini-Prato described the successful use of a porcine derived, two-layer xenogenic collagen matrix (Mucograft<sup>®</sup>, Geistlich) in the treatment of 11 maxillary teeth with gingival recession in three non-smoking women.<sup>54</sup> Cardaropoli *et al* reported that collagen matrix had more gingival recession reduction than CAF alone in a randomized

controlled clinical trial in 32 patients with 113 Miller Class I and II gingival recession defects.<sup>55</sup>

Extracellular matrix (ECM) materials have indications for a variety of procedures including increasing keratinized tissue width, root coverage, guided tissue regeneration, and guided bone regeneration. DynaMatrix<sup>®</sup> (Keystone Dental) is a graft material from the submucosal tissues of the small intestine in pigs. DynaMatrix Plus<sup>®</sup> is a double layered version of DynaMatrix<sup>®</sup> made specifically for the treatment of gingival recession defects. The product is indicated to be used in conjunction with a CAF. The manufacturer processes it in such a way that retains the natural composition of matrix molecules like collagen types I, III, IV, and VI, glycosaminoglycans, glycoproteins, proteoglycans, and growth factors.<sup>57</sup> Nevins *et al* investigated ECM in a randomized clinical trial as a potential alternative to autogenous free gingival grafts (FGG) in augmenting keratinized tissue width.<sup>56</sup> The authors reported no statistically significant differences in probing depth, vertical gingival recession, horizontal gingival recession, and keratinized tissue width between FGG and ECM.

To the author's knowledge, there are no randomized clinical trials that investigate the use of DynaMatrix Plus<sup>®</sup> (Keystone Dental) in the treatment of gingival recession. DynaMatrix Plus<sup>®</sup> is a double layered version of DynaMatrix<sup>®</sup> made specifically for the treatment of gingival recession defects. The purpose of this study is to compare mean percent root coverage and clinical attachment levels in areas of localized marginal tissue recession between DynaMatrix Plus<sup>®</sup> and CTG in a randomized clinical trial.

#### **II.2** Materials and Methods

This study was approved by the Texas A&M University Baylor College of Dentistry institutional review board and undertaken as a randomized, prospective clinical trial. The informed consent form was approved by the IRB for use in this study.

A power analysis (G\*Power 3.1.2) was performed before initiating the study. A two-tailed t-test determined that 15 samples for test and control (total sample of 30) was required to determine a difference of 0.5 mm between groups ( $\alpha = 0.05$ , 1 -  $\beta = 0.8$ ).

#### **II.2.1** Patient Population

Eighty-five subjects were screened from February 2013 to June 2014 from the BCD general patient pool. Inclusion criteria included:  $\geq$  18 years old; buccal, vertical recession (VR) defects of  $\geq$  2 mm (measured from the CEJ to the midfacial gingival margin) limited to incisors, canines, and premolars only; Miller Class I, II, and III recession defects; adequate plaque control (modified O'Leary index <sup>60</sup> of  $\geq$ 85% prior to surgical therapy); vital or non-vital teeth; probing depths of  $\leq$ 3 mm; presence or absence of bleeding on probing. Unilateral and bilateral sites of gingival recession were included in the study. Patients who presented with a unilateral site of gingival recession were randomly assigned via a computer generated randomized list to receive either ECM (DynaMatrix Plus<sup>®</sup>) or CTG. Assignment was given on the day of the surgical appointment to limit bias. If a patient presented with bilateral sites of gingival recession in contralateral quadrants of the same arch or different arch, then both were treated in a split-mouth design. In these instances, assignment of graft material was determined by coin toss. Both sites were treated at the same appointment in 6 of the 8 split-mouth

patients. A single site or multiple contiguous sites of gingival recession were also included in the study. If multiple contiguous sites were treated, then only one tooth that meets the criteria above and with the greatest amount of VR was included for this study.

Subjects could not participate in the study based upon the following exclusion criteria: smoke more than ten cigarettes per day or use nicotine replacement therapy, allergy to iodine or shellfish, had previous surgery performed at the surgical site, non-English speakers, uncontrolled or poorly controlled systemic conditions, pregnant or lactating females, use of immunosuppressant medications, existing buccal or facial restoration on the qualifying tooth.

All subjects received a prophylaxis and scaling, if necessary, prior to surgery. Oral hygiene instructions were given to address habits related to the etiology of gingival recession and to demonstrate effective plaque control. Prior to surgery, photographs and periapical radiographs were taken of the selected teeth. All participants received verbal and written instructions and signed an informed consent document prior to enrolling into the study.

## **II.2.2** Clinical Parameters

Evaluation of the following clinical parameters immediately prior to surgery (baseline), and at 3, and 6 months post-operatively included: 1) vertical recession (VR) measured as the distance from the cementoenamel junction (CEJ) to the free gingival margin (FGM) in mm; 2) horizontal recession (HR) measured at the CEJ in mm; 3) probing depth (PD) on midfacial aspect measured as the distance from the FGM to the bottom of the sulcus in mm; 4) clinical attachment level (CAL) measured as the distance

from the CEJ to the bottom of the sulcus in mm; 5) presence or absence of bleeding on probing (BOP) on midfacial aspect; 6) papillary height mesial and distal (PHM, PHD), defined as the distance from the tip of papilla to the base of the papilla at level of the CEJ; 7) papillary width mesial and distal (PWM, PWD) measured at the base of papilla at CEJ level for both the mesial and distal papilla adjacent to the recession; and 8) the width of the keratinized tissue (KT) from the FGM to the mucogingival junction in mm. Lugol's solution (Sigma-Aldrich) applied to the patient's gingiva and alveolar mucosa using a cotton tip applicator helped identify KT and alveolar mucosa. A stent was fabricated from the patient's diagnostic cast to aid in the measurements of VR and PD. Intraoperative measurements of each patient included: 1) facial flap thickness (FT) at the midfacial aspect; 2) distance from the CEJ to the alveolar bone (BH) at the midfacial aspect.

All clinical parameters VR, HR, PD, CAL, BOP, PH, PW, KT, FT, and BH were measured with a University of North Carolina periodontal probe to the nearest 0.5 mm. Two blinded periodontists (DGK, JAR) performed all clinical measurements. When the CEJ was obliterated by a non-carious cervical lesion, the most coronal aspect of a noncarious cervical lesion served as a reference point for VR measurements.

## II.2.3 Surgical Procedure

The surgical procedure was identical for both control and test groups with the only difference being the graft material. The surgical sites were anesthetized, and the exposed root surfaces were thoroughly scaled and root planed to remove plaque and create a smooth surface. The exposed root surfaces on the control and test side were conditioned with 24% ethylenediaminetetraacetic acid (EDTA, PrefGel<sup>®</sup>, Straumann) gel for 2 minutes to remove the smear layer and attempt to ensure a biocompatible surface. The sites were rinsed with saline solution. Incision design followed a technique previously described by Zucchelli and De Sanctis (Fig 2A).<sup>40</sup> Grafts were trimmed so that it completely covered the defect and extended at least 3 mm beyond the osseous defect margins, and positioned at the CEJ. Grafts were secured against the root surface via 5-0 chromic gut sling suture (Fig 2B). The flap was coronally advanced to cover the entire graft and secured with 6-0 polypropylene (Prolene<sup>®</sup>, Ethicon) suture using a sling suture technique (Fig 2C). Additional interrupted sutures were placed as needed to correctly position the papilla. CTG was harvested from the palate in a surgical approach described by Reiser *et al* in the control group only (Fig 2D ).<sup>51</sup>

## II.2.4 Post-Surgical Care

Each patient took 500 mg amoxicillin, three times daily for 7 days, or 300 mg clindamycin, three times daily for 7 days, if the patient was allergic to penicillin. In addition, 5 mg hydrocodone with 325 mg acetaminophen was prescribed for post-operative analgesia. Instructions were given for ice pack application immediately after surgery on an intermittent basis for the first 3 to 4 hours. All patients were advised to replace mechanical oral hygiene measures in the surgical sites with chlorhexidine gluconate 0.12% rinse for 3 weeks, and instructed to use a liquid diet for the first 48 hours followed by a soft diet for the next 3 weeks. Sutures were removed by 3 weeks. Gentle tooth brushing (roll technique) was resumed after suture removal and continued

for an additional 2 weeks. Professional plaque control was performed at the 1 week, 3 weeks, 3 months, and 6 months recall appointments.

#### II.2.5 Statistical Analysis

All variables measured at baseline, 3, and 6 months post-operatively were analyzed with a longitudinal approach for nonparametric data according to treatment group (CTG or ECM) and Miller Classification, assuming an unstructured covariance matrix, and a mixed effect between time and the variable of interest. BOP was measured as odds ratio for the longitudinal data analysis. Time was treated as an ordinal variable as opposed to a continuous linear variable since not all patients were measured at the same time. FT and BH were compared using two-sample tests for comparison between groups. All tests were compared with  $\alpha = 0.05$ . For all described tests, statistical significance was achieved when p < 0.05.

## **II.3 Results**

One patient treated with CTG was lost to dropout. Another patient was removed from the analysis because of the patient's inability to conform to study protocols. This patient received split-mouth treatment and was diagnosed with Miller Class III defects. Twenty patients (15 females, 5 males, aged 22 to 69 years; mean age: 51.4 years) with a total of 28 defects completed the study. There were 5 Miller Class I, 3 Miller Class II, and 20 Miller Class III defects which consisted of 7 incisors, 5 canines, and 16 premolars. Thirteen sites were treated with CTG (control) while 15 sites were treated with ECM (test). Eight patients had contralateral recession defects and were treated bilaterally with either CTG or ECM.

Results were tabulated and analyzed as described above using SAS 9.3, in particular prewritten functions such as proc mixed with proc ranked to use Friedman's method, proc glimmix (for BOP), and proc univariate for BH and FT. In a longitudinal study, the purpose is to test for outcome as a function of time, and to determine if there is a significant difference between treatment groups overall. To test this, a test of interaction is required, followed by an analysis of the individual variables. In the analysis, at an  $\alpha = 0.05$ , interaction is tested between the variable of interest and time (baseline, 3 months, 6 months): then each individual variable is tested.

 $H_0$ : There is no interaction effect for the variable in consideration.

 $H_A$ : There is an interaction effect for the variable in consideration.

If this test rejects the null (i.e. p < 0.05 in the above hypothesis test), then the test for treatment effect is as follows:

 $H_0$ : There is a treatment effect for the variable in consideration.

 $H_A$ : There is no treatment effect for the variable in consideration. and for time:

 $H_0$ : There is no time effect for the variable in consideration.

 $H_A$ : There is a time effect the variable in consideration.

In SAS, it is tested as a Type III test for effects, and is tested compared to an F distribution. Therefore, when p < 0.05 then the null hypothesis is rejected and the difference between the test and control groups is significant.

The Wilcoxon-Mann-Whitney ranked sum test corresponds to a two-sample ttest: the difference between the two tests is that Wilcoxon-Mann-Whitney test applies for nonparametric data (i.e. data that does not follow a known distribution) versus a parametric distribution, as the *T*-distribution requires.

Baseline, 3 months, and 6 months clinical measurements for test and control groups are summarized in Table 1.

#### **II.3.1** Vertical Recession

Progression of VR in both groups over time is summarized in Figure 2A. In general, both CTG and ECM had significant improvement in VR when compared to baseline (p=0.0027). Baseline VR showed no significant difference between groups (2.81±0.663 mm for CTG and 2.73±0.594 for ECM). At 3 months, remaining VR decreased in both groups (0.46±0.660 mm for CTG and 1.07±0.678 mm for ECM). At 6 months, the CTG treated sites increased to 0.69±0.879 mm, whereas the ECM treated sites decreased to 0.97±0.694 mm. At 6 months, CTG treated sites had greater improvement in VR than ECM, but the difference was not considered statistically significant. Although a significance was reached when time and treatment groups were analyzed simultaneously (p=0.0180), significance was not maintained when data was analyzed between groups alone (p=0.1296). As seen in Figure 2A, similar trends were found when Miller Class III defects were examined separately. Again, the difference in VR between groups was not significant between groups over time.

### II.3.2 Horizontal Recession

There were no significant differences over time between groups in HR (p=0.1487). Baseline mean HR was  $3.54\pm1.010$  mm in CTG and  $3.70\pm0.922$  mm in ECM. At 3 months, HR decreased to  $1.38\pm1.770$  mm in CTG and  $2.63\pm1.343$  mm in

ECM. At 6 months, HR increased to  $1.65\pm1.599$  mm in CTG and  $2.67\pm1.305$  mm in ECM. Overall, there was a significant decrease in HR in both groups.

#### *II.3.3 Probing Depth*

Progression of PD in both groups over time is summarized in Figure 2B. At baseline, the PD for CTG and ECM were  $1.46\pm0.519$  mm and  $1.47\pm0.481$  mm, respectively. At 3 months, PD decreased in both CTG and ECM to  $1.27\pm0.525$  mm and  $1.10\pm0.387$  mm, respectively. At 6 months, PD increased to  $1.31\pm0.384$  mm for CTG and decreased to  $1.07\pm0.176$  mm for ECM. Similar results were noted when Miller Class III data was analyzed separately. It should be noted that CTG had a higher PD than ECM at both 3 months and 6 months. The same was found when Miller Class III defects were analyzed separately. However, all intergroup differences did not reach statistical significance in all data and in Miller Class III defects alone.

## II.3.4 Clinical Attachment Level

Progression of CAL in both groups over time is summarized in Figure 2C. CAL improved significantly for both groups between baseline and 6 months (p < 0.0001). The mean CAL at baseline for CTG and ECM was  $4.27\pm0.992$  mm and  $4.20\pm0.797$  mm, respectively. At 3 months, CAL decreased to  $1.73\pm0.633$  mm and  $2.13\pm0.743$  mm for CTG and ECM, respectively. At 6 months, CAL increased in CTG to  $2.00\pm0.913$  mm and decreased in ECM to  $2.00\pm0.732$  mm. Difference in mean CAL between CTG and ECM was statistically significant at 3 months only. When treatment group and time were analyzed globally, no significant interaction or difference was found (p=0.2854). *II.3.5 Keratinized Tissue* 

Progression of KT in both groups over time is summarized in Figure 2D. There was a statistically significant increase in mean KT between baseline and 6 months for both groups (p=0.0038). At baseline, mean KT was  $1.88\pm1.261$  mm and  $1.60\pm0.870$  mm for CTG and ECM, respectively. At 3 months, KT increased to  $2.19\pm0.925$  mm for CTG and  $2.23\pm0.923$  mm for ECM. At 6 months, mean KT decreased slightly to  $2.15\pm1.125$  mm for CTG, and increased slightly to  $2.33\pm1.063$  mm for ECM. ECM had a greater amount of KT gain than CTG. However, intergroup difference over time was not significant (p=0.2228).

#### **II.3.6** Papillary Measurements

Papillary measurements are summarized on Table 1. Generally, PHM, PHD, PWM, PWD decreased in both groups over time. The difference between baseline and 6 months did not reach statistical significance within each group. Furthermore, the difference between groups was not significant at any time point.

## II.3.7 Bleeding on Probing

After reviewing data on BOP, it was apparent that the model did not converge even with the use of pseudocounts. This is due to the uniformity of the data (lack of variability); therefore, all analysis as part of this variable was not completed. To solve this, more data is needed in order to increase variability.

## **II.3.8** Miller Classification

The Miller Classification variable is an ordinal variable, where a score of 1 is less severe than 2 and 3; a score of 2 is more severe than 1 and less severe than 2; and 3 is the most severe Miller score. The appropriate test for this kind of ordinal data is a Wilcoxon Ranked sum test. The test concluded that at  $\alpha$ =0.05 with a *p*=0.1659 there was no significant difference between the treatment groups in regards to Miller score. For this analysis, dropouts were considered because this particular test was based on baseline only.

#### II.3.9 Flap Thickness

Mean FT was  $1.05\pm0.31$  mm for all treated sites. FT was shown not to belong to any distribution; therefore, a nonparametric test was used to compare FT between each group. The Wilcoxon Ranked Sum Test revealed that there was no significant difference between treatment groups compared to an  $\alpha = 0.05$  and a p=0.5256. This analysis was also used to see if there was a difference in FT between the two groups at Miller Class III, which also revealed that there was no significant difference between the treatment groups (p=0.6644).

## II.3.10 Alveolar Crest to CEJ

Mean BH was 5.68±1.65 mm for all treated sites. A distribution analysis verified that BH followed closely to a log-normal distribution ( $\alpha$ =0.05, p=0.21). Subsequently, a t-test with the established distribution as log-normal was used to test if there was a difference between the study groups. At an  $\alpha$ =0.05, there was no difference in BH between the groups at baseline for both the entire data (p=0.8245) and Miller Class III data (p=0.8256).

# CHAPTER III

## CONCLUSION

Finding a material that decreases post-operative morbidity and maintains the effectiveness of CTG in root coverage is a goal for many clinicians. ECM allows the clinician to avoid a second surgical site at the palate, thus reducing both post-operative morbidity and surgery duration. ECM provides a limitless supply of graft material for use in the treatment of gingival recession.

Greenwell *et al* explained that current reporting trends of root coverage are deceptive because only the amount of soft tissue covering the original defect is calculated.<sup>35</sup> For example, a tooth with 6 mm of VR is treated, and VR decreased to 2 mm. Current trends report that 66% of root coverage was achieved. Current trends in reporting root coverage should include a name change to defect coverage to appropriately explain the measurement.

% Defect Coverage =  $(1 - VR_t / VR_0) \times 100$ 

 $VR_0 = baseline VR$ ,  $VR_t = VR$  at time t.

Furthermore, Greenwell *et al* recommends describing the root coverage as a function of VR at baseline and after treatment over root length, i.e. a 6 mm of VR on a 12 mm length root would have 50% root coverage. Since the actual root length of every tooth that is treated is often unknown, Greenwell *et al* recommends a generally accepted universal root length of 13.63 mm.<sup>36</sup>

% Root Coverage =  $100 \times (13.63 - VR_t) / 13.63$ 

See Table 2 for a summary of percent root coverage in test and control groups over time according to criteria set by Greenwell *et al*. To illustrate the differences in reporting trends, see Table 3 for a summary of percent defect coverage.

Greenwell *et al* states that successful defect elimination is achieved when 95 to 100% mean root coverage was obtained, and 90% coverage was obtained 90% of the time.<sup>35</sup> As can be seen in Table 3, 71.65% and 70.63% defect coverage was obtained post-treatment in control and test, respectively. This could be due to the inclusion of 20 Miller Class III defects in the study design. Miller explained that complete root coverage was not usually achieved on teeth that have interproximal bone loss or that have extruded, i.e. Class III and Class IV defects, due to the fact that a graft cannot be maintained at the CEJ of the tooth as this would be "physically impossible".<sup>31</sup>

The authors recommend that the definition of tooth malposition should specify if the tooth is positioned in such a way that the root is outside of the alveolus. A labioverted tooth may have a diminished root coverage outcome. Blood supply from the periodontal ligament, alveolar bone, and supraperiosteal vessels are compromised often in these Miller Class III defects due to malposition. Conversely, a tooth that is simply rotated is considered malposed but does not necessarily pose a threat to the root coverage outcome because it usually has better supraperiosteal and periodontal ligament blood supply.

The authors observe that Miller Class III defects may be under-reported in the literature due to the overlooked criteria that tooth malposition alone would indicate a Miller Class III. Twenty of the 28 teeth analyzed in this study were Miller Class III

recession defects because of tooth malposition and/ or interproximal bone loss. This study included Miller Class III defects due to the substantial prevalence of these defects among individuals with gingival recession. Marini *et al* found that 89% of the 380 adult subjects exhibited at least one dental surface with gingival recession.<sup>17</sup> Specifically, 3,526 teeth and 6,123 surfaces had gingival recession. The majority of these defects were Miller Class I and III defects, (59% and 33% respectively).

Interestingly, Miller Class III defects treated with ECM demonstrated no significant difference in remaining VR and CAL than defects treated with CTG. Testing for material effectiveness in gingival recession treatment is often conducted in Miller Class I and II defects. On the contrary, Miller Class III defects are less predictable in achieving successful root coverage and thus may serve as a better gauge of material effectiveness. Typically, clinicians would elect for CTG in the treatment of Miller Class III defects since it is the current gold standard in graft material. However, this study demonstrated that ECM was as effective as CTG in the treatment of Miller Class III defects.

Other studies that included Miller Class III defects report similar findings to the current study. Sang Ho Shin *et al* treated 42 Miller Class I and 40 Miller Class III defects in 14 patients, and reported mean defect coverage of 73.4% for ADM and 79.4% for ADM plus enamel matrix derivative (EMD).<sup>61</sup> Remaining VR at 6 months was 0.94±0.78 mm for ADM and 0.81±0.58 mm for ADM plus EMD. Mean root coverage was 93.1% for ADM and 94.1% for ADM plus EMD when using the Greenwell *et al* criteria for root coverage. Barker *et al* treated 44 Miller Class I and 8 Miller Class III

defects in 14 patients, and reported 81.4% and 83.4% mean defect coverage for acellular dermal matrices AlloDerm<sup>®</sup> and Puros<sup>®</sup> Dermis, respectively.<sup>62</sup> Remaining VR at 6 months was 0.65±0.76 mm for AlloDerm<sup>®</sup> and 0.67±0.76 mm for Puros<sup>®</sup> Dermis. Mean root coverage was 95.2% for AlloDerm<sup>®</sup> and 95.1% for Puros<sup>®</sup> Dermis when using the Greenwell *et al* criteria for root coverage. Carney *et al* treated 24 Miller Class I or II defects and 16 Miller Class III defects, and reported mean defect coverage of 76.7% and 69% for ADM and ADM plus recombinant human platelet-derived growth factor (rhPDGF), respectively.<sup>63</sup> When analyzed separately, Miller Class III defects demonstrated mean defect coverage of 60.8% and 51.5% for ADM and ADM plus rhPDGF, respectively. Remaining VR at 6 months was 0.76±0.84 mm for ADM and 0.65±0.76 mm for ADM plus rhPDGF. Mean root coverage was 94.4% for ADM and 95.2% for ADM plus rhPDGF when using the Greenwell *et al* criteria for root coverage.

Based on the results of this randomized clinical trial, there were no statistically significant differences in VR and CAL between test and control groups over time in the treatment of gingival recession defects. Evidence from this study supports the use of ECM in root coverage procedures in Miller Class I, II, and III defects. Because only one ECM product was tested in this study, the conclusions drawn can only be applied to the use of this specific product. Future studies should examine and evaluate histologic differences in root attachment between CTG and ECM. Furthermore, long term follow-up studies should be conducted, ideally with larger sample sizes.

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# APPENDIX A

# TABLES

Table 1

# Mean ( $\pm$ SD) of Variables at Baseline and at 3 and 6 Months

	Baseline (mm)			3 Months (mm)				6 Months (mm)				
	Control		Test		Control		Test		Control		Test	
VR	2.81	<u>+</u> 0.663	2.73	<u>+</u> 0.594	0.46	<u>+</u> 0.660	1.07	<u>+</u> 0.678	0.69	<u>+</u> 0.879	0.97	<u>+</u> 0.694
HR	3.54	$\pm 1.010$	3.70	<u>+</u> 0.922	1.38	<u>+</u> 1.770	2.63	<u>+</u> 1.343	1.65	<u>+</u> 1.599	2.67	<u>+</u> 1.305
PD	1.46	<u>+</u> 0.519	1.47	$\pm 0.481$	1.27	<u>+</u> 0.525	1.10	<u>+</u> 0.387	1.31	<u>+</u> 0.384	1.07	<u>+</u> 0.176
BOP	0.15	<u>+</u> 0.376	0.33	±0.488	0.00	$\pm 0.000$	0.27	<u>+</u> 0.458	0.00	$\pm 0.000$	0.13	<u>+</u> 0.352
CAL	4.27	±0.992	4.20	$\pm 0.797$	1.73	<u>+</u> 0.633	2.13	±0.743	2.00	$\pm 0.913$	2.00	<u>+</u> 0.732
КТ	1.88	$\pm 1.261$	1.60	±0.870	2.19	<u>+</u> 0.925	2.23	±0.923	2.15	$\pm 1.125$	2.33	$\pm 1.063$
РНМ	3.42	<u>+</u> 0.787	3.47	<u>+</u> 0.442	3.00	<u>+</u> 0.842	3.20	<u>+</u> 0.775	3.04	<u>+</u> 0.776	3.20	<u>+</u> 0.561
PHD	2.88	<u>+</u> 0.583	3.17	<u>+</u> 0.588	2.62	<u>+</u> 0.740	2.73	<u>+</u> 0.998	2.65	<u>+</u> 0.625	3.00	<u>+</u> 0.707
PWM	3.42	<u>+</u> 0.703	3.70	<u>+</u> 0.592	3.04	<u>+</u> 0.749	3.20	<u>+</u> 0.621	3.12	<u>+</u> 0.712	3.10	<u>+</u> 0.471
PWD	3.46	<u>+</u> 0.923	3.60	<u>+</u> 0.573	2.96	$\pm 1.050$	3.00	$\pm 1.102$	3.19	±1.052	3.13	<u>+</u> 0.694

# Table 2

Percent Root Coverage at Baseline and at 3 and 6 Months\*

Base	eline	3 Mo	onths	6 Months		
Control	Test	Control	Test	Control	Test	
79.82%	79.26%	94.36%	93.93%	93.93%	93.65%	

\* % Root Coverage = 100 x (13.63 - VR<sub>t</sub>) / 13.63 ; VR<sub>t</sub> = VR at time t. Greenwell *et al* criteria.

Table 3

Percent Defect Coverage at 3 and 6 Months\*

3 Mo	nths	6 Months			
Control	Test	Control	Test		
74.09%	72.30%	71.65%	70.63%		

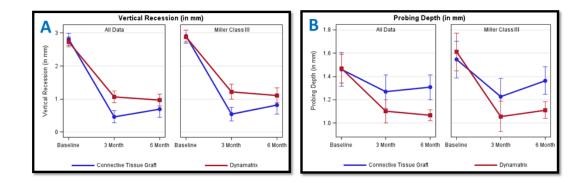
\* % Defect Coverage =  $(1 - VR_t / VR_0) \times 100$ ;  $VR_0$  = baseline VR,  $VR_t$  = VR at time t.

## APPENDIX B

# FIGURES

Figure 1

Clinical Parameters Over Time



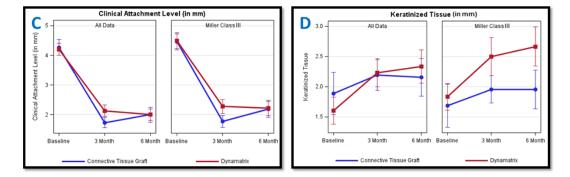


Figure 2

Surgical Procedure



Figure 3

Clinical Results Over 6 Months







