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Acellular Dermal Matrix Combined with a Coronally Advanced Flap in the Treatment of Gingival Recession in Thin versus Thick Biotype Population: A Controlled Clinical Trial

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ABSTRACT

Aim and Hypothesis

Gingival recession is a common defect of the periodontal soft tissues, whose prevalence has been estimated between 50% and 70% of the population. Gingival recession is more common in individuals with thin biotype than those with thick biotype. While an autogenous connective tissue graft is the gold standard, acellular dermal matrix (ADM) is an acceptable alternative material for the treatment of such a defect. The present study aimed to investigate the impact of gingival biotypes on the outcomes of root coverage procedures performed with ADM. The hypothesis in this study is that the thick biotype group will achieve higher percentage of root coverage at 3 months.

Materials and Methods:

The study included 10 patients with thick and 11 with thin biotype. Each patient had two adjacent recession defects. Thick biotype was defined as gingival thickness≥0.8 mm while gingival thickness <0.8 was considered a thin biotype. In both groups, a coronally positioned flap was utilized to cover a standard sized piece of ADM over the defects. Clinical measurements including recession height (RH) and width (RW), gingival thickness (GT), keratinized tissue width (KTW), probing depth (PD), bleeding on probing (BOP) and clinical attachment level (CAL), were measured at baseline and at 3 months follow up. A patient survey evaluating sensitivity, esthetics and pain was collected at baseline and at 1 week, 3 weeks and 3 months follow-up visits.

For between-group analysis, the independent samples t-test was used if the data was normally distributed, while the Mann-Whitney U test was used if the data were not normally distributed. For within-group analysis comparing baseline and 3-month data, the paired samples t-test or the Wilcoxon signed-rank test were used if the data were not normally distributed. Alpha was set at 0.05 to obtain a power of 99%. Dentinal hypersensitivity was compared between groups at baseline, and the changes in sensitivity at 3 weeks and 3 months were also compared between groups. Similarly, complication rates were compared between the two groups at 1 and 3 weeks post-surgery.

Results: Between baseline and at 3 months, both groups achieved statistically significant reduction in RH (thick: from 2.63±0.57 mm to -0.02±1.47 mm , p <0.001; thin: from

2.62±0.33 mm to 0.18±1.18 mm, p <0.001) and RW (thick: from 3.30±0.62 mm to 0.00±0.62 mm, p = 0.001; thin: from 3.80±1.44 mm to 0.72±2.22 mm, p<0.001). The differences in the reduction between the groups was statistically not significant (RH: p=0.480; RW: p =0.557). The differences in terms of percent root coverage, either capped (thick: 100.00±28.00%, thin: 92.36±62.80%, p = 0.282) or non-capped (thick: 115.16±54.68%, thin: 95.12±14.7%, p = 0.387), was found to be statistically non-significant. At three months, the comparison between the groups yielded a significant difference between groups for KTW, (thin: 1.58 ± 0.62 mm versus thick: 2.49 ± 0.96 mm, p = 0.017), and GT (thin: 1.21 ± 0.30 mm versus thick: 1.58 ± 0.20 mm, p = 0.004). Within the groups, differences for GT (thin: from 0.59±0.14 to 1.21±0.30), the CAL (thin: from 4.48±0.74 to 3.38±1.21; thick: 4.65±0.97 to 2.22±1.41) were judged to be statistically significant, (p=0.003 and p=0.02 for GT and CAL respectively). The frequencies for complete root coverage, were 63.6% and 80% in the thin and the thick groups respectively. Baseline comparison between dentinal hypersensitivity scores was not significant (thin: 5±4; thick: 3±5 p=0.847). Similarly, the changes in dentinal hypersensitivity between baseline and 3 weeks, (thin: 1±4; thick: 3±4, p=0.193), 3 weeks and 3 months, (thin: 0±4 and thick: 0±1; p=0.056), and baseline and 3 months (thin: 3±5; thick: 2±3, p=0.562), were not statistically significant. Since the data was not normally distributed, the Mann Whitney U-test was used to compare groups. Complication rates at one and three weeks yielded no statistically significant difference between groups, (one week: thin: 40%; thick: 27.3%, p=0.659; three weeks: thin: 20%; thick: 27.3%, p=1.00). Chi-square test was used to compare complications between groups.

Conclusions: Both groups in the present study achieved a high degree of root coverage and were associated with a high frequency of complete root coverage at the three-month follow-up. The gingival thickness increased by a similar degree in both groups and at three months, both had a gingival thickness greater than 0.8 mm. Within the limits of the study, it is concluded that ADM is effective in treating moderate multiple gingival recessions for both biotypes. As a side benefit, it thickens the gingival tissue and may convert a thin biotype to a thick one.

DEDICATION

To my parents, who have supported and encouraged me throughout
my long professional and educational endeavors.
Ad astra per aspera!

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LIST OF ABBREVIATIONS

ADM: acellular dermal matrix

EMD: enamel matrix derivatives

CAF: coronally advanced flap

CTG: connective tissue graft

PI: Plaque Index

GI: Gingival Index

RH: recession height

RW: recession width

GT: gingival thickness

PD: probing depth

CAL: clinical attachment level

KTW: keratinized tissue width

BOP: bleeding on probing

CEJ: cemento-enamel junction

FGM: free gingival margin

GTR: guided tissue regeneration

MAGR: multiple adjacent gingival recessions

MCAF: modified coronally advanced flap

PRF: platelet rich fibrin

TnB: Thin gingival biotype group

TkB: Thick gingival biotype group

LIST OF SYMBOLS

Acellular Dermal Matrix Combined with a Coronally Advanced Flap in the Treatment of Gingival Recession in Thin versus Thick Biotype Population:

A Controlled Clinical Trial

Introduction

The Glossary of Periodontal Terms [2] from the American Academy of Periodontology, (2001), defines the gingiva as “the fibrous investing tissue, covered by keratinized epithelium, that immediately surrounds a tooth and is contiguous with its periodontal ligament and with the mucosal tissues of the mouth.” The gingiva may further be divided in “free” and “attached” gingiva.

The keratinized gingiva extends apically to the mucogingival junction. Attached gingiva is firmly attached to the underlying bone and cementum by connective tissue fibers, which may give it a distinctive orange peel appearance known as “stippling” [3].

The free gingiva is coronal to the attached gingiva, and as the name suggests it is movable. The tissue follows the tooth shape and contour, terminating coronally with the free gingival margin. Interproximally the free gingiva extends between the teeth, to form the interdental gingiva or interdental papillae, whose shapes depend on the contact relationships between teeth, the width of the approximal tooth surfaces and the course of the cemento-enamel junction. In a fully erupted tooth, the free gingival margin is usually located on average 1.5-2 mm coronal to the cemento-enamel junction. [3]

Gingival recession is defined as the displacement of the free gingival margin apically to the cemento-enamel junction, which results in exposure of the root surface [4]. According to the Glossary of Periodontal terms, a distinction may be made between two separate entities, “gingival recession” namely the “location of the gingival margin apical to the cement-enamel junction”, and “surgical recession”, defined as the “location of the marginal tissues apical to the cemento-enamel junction as a result of periodontal surgery”. [2]

Large epidemiologic studies of gingival recessions have reported a high prevalence of recession worldwide. Although studies conducted on different ethnic groups have reported differences in prevalence, there seemed to be some common trends. Recession was found to be more prevalent with increasing age, and buccal surfaces are most commonly affected. Furthermore, recession was for the most part associated with male gender. [5] A cross-sectional study published in 1999 by Albander and King, [6]investigating the prevalence and extent of gingival recession, bleeding and calculus accumulation in the American population showed that the prevalence of recession defects ≥ 1 mm was 22% in persons of age 30 or above. The study also confirmed that as the age of the subjects increased, the extent and prevalence of recession defects also increased. The age group between 30 and 38 years of age had a prevalence of 37.8%, whereas the 80-90 age group showed a prevalence of 90.4%, representing a significant increase compared to the previous age group. The most frequently involved teeth were the maxillary molar and the mandibular central incisor. Within each age, gender and ethnic group the buccal sites always exhibited a higher prevalence of recession defects compared to the mesial sites.

In their classic 1968 article, Sullivan and Atkins [7] classified recession defects subjectively into four categories, namely deep wide, deep narrow, shallow wide and shallow narrow. These categories were based upon the possibility of predictably obtaining root coverage of the exposed root surface through the phenomenon of bridging. This is the biological process through which the grafted tissue will survive on the avascular root surface thanks to the vascularization it received from the adjacent vascular bed. The amount of bridging and hence the graft survival depends upon the width of the root surface and the vascularity of the donor tissue and recipient bed. Intuitively, the most difficult defects to treat are those in the deep wide category, because the width of the avascular surface is too great for two-point collateral circulation, from the mesial and distal borders of the vascular bed to predictably bridge the coronal graft tissue. Similarly, the area apical to the graft will be too far away to aid in vascularizing the graft tissue and, as a result, necrosis will occur. Treatment of shallow wide defects is associated with a better prognosis because of three sources of circulation, (from the apical as well as the mesial and distal borders of the adjacent bed). In both deep and shallow narrow defects, graft survival may be enhanced due to two sources of circulation from the mesial and distal margins.

The most commonly used classification system of gingival recession is that presented by Miller [8], which divides recession defects into four categories depending on their severity and prognosis. A class I gingival recession does not extend past the mucogingival junction. A class II Miller is a recession defect that extends past the mucogingival junction but that does not have interproximal loss of attachment. A class III defect has loss of interproximal attachment or tooth rotation. A class IV defect has severe interproximal loss of attachment and the recession defect extends past the mucogingival junction. According to the original article [8], Miller class I and II defects generally have a good prognosis when treated with free gingival grafts but Miller class III and IV defects have a less predictable response to therapy. In Miller class IV defects, loss of interproximal tissue is severe, complete root coverage occurs only occasionally and cannot be predicted.

Although the Miller classification system is still widely used, it has been criticized due to its shortcomings in certain clinical scenarios. These include the distinction between Miller I and II defects when the recession defect passes the mucogingival junction but still conserves a band of keratinized tissue. Although not important from a prognostic viewpoint, the presence of keratinized tissue may influence the choice of surgical technique. Furthermore, the method of determining interproximal attachment loss to discriminate between Miller III and IV defects is unclear, as is the impact and degree of tooth malposition on the prognosis.

The prognostic indications of the Miller classification depend on the amount of root coverage that may be achieved with a free gingival graft. Currently other treatment options are preferred when root coverage is the main goal, and it has been suggested that the Miller classification may be outdated as it no longer reflects the true root coverage potential that may be achieved with other therapeutic modalities.

According to the Miller classification, root coverage is unpredictable in class III defects. However, Aroca et al. [9] challenged this concept. In their study, multiple adjacent Miller class III recessions were treated with a connective tissue graft in combination with a tunnel technique. The test group was additionally treated with EMD. The results showed a high incidence of complete root coverage, (8 cases out of 20, corresponding to 40%), and no differences were reported between test and control sites, which seemed to question the benefit of adding EMD to the surgical area. The authors concluded that a connective tissue graft (CTG) in association with a tunnel procedure would yield predictable outcomes in the treatment of multiple Miller class III recessions.

Criticisms of the Miller classification and prognosis system also arise from the fact that it does not address cases of noncarious cervical lesions with non-identifiable CEJ. This issue is of critical importance as it is not possible to evaluate the outcome of a given root coverage procedure correctly if the extent of the recession, (measured from the CEJ), cannot be determined. [10]

Alternative classification systems have been proposed to overcome the limitations of the Miller classification. For example, Ozcelik et al. [11] suggested quantifying the amount of avascular exposed root surface area, (AERSA). According to these authors, the amount of exposed root structure is more important in predicting the amount of root coverage that may be achieved, as opposed to the interproximal recession, which is the crucial aspect in the Miller classification. The results of the study by Ozcelik showed that AERSA was strongly correlated with mean root coverage, and that the amount of exposed root structure was inversely correlated with complete root coverage. Interestingly, the study also underlined the importance of tissue thickness at baseline as a prognostic factor for complete root coverage.

Cairo et al. [12] have recommended a classification system based on the relationship between interproximal and proximal attachment loss and thus divide recession defects into 3 categories. These are RT1: gingival recession with no interproximal attachment loss; RT2: interproximal attachment loss is present but does not exceed the amount of buccal recession; RT3: interproximal loss of attachment higher than the amount of buccal recession. In the aforementioned classification system, the amount of buccal attachment loss was measured from the buccal CEJ to the bottom of the buccal pocket, whereas interproximal loss of attachment was measured as the distance between the interproximal CEJ and the depth of the interproximal pocket. The authors show that the CAL may be used to classify gingival recession defects and that the amount of interproximal attachment is associated with the expected degree of root coverage. RT1 defects showed a higher degree of root coverage than RT2 defects, while RT3 defects were treated for gingival augmentation and not root coverage.

The limitations of the Miller classification system are stressed by the fact that the horizontal component of the recession defects is not taken into account, although the importance of this parameter is well-known, as previously indicated by Sullivan and Atkins.[7]

The etiology of gingival recession is complex and various possible causes have been identified. Vigorous and aggressive toothbrushing, [13] orthodontic treatment with labial movement of teeth out of the bony housing of the alveolar process, iatrogenic factors, (plaque retentive prosthetic restorations or restorations impinging on the biologic width, inadequate and compressive removable partial dentures) [14], plaque accumulation and periodontal disease [4], incisal relationship causing trauma and subsequent recession of the free gingival margin [15] as well as malalignment [16] have all been considered etiologic factors in the development of gingival recession. Furthermore, as explained by Smith, [17] developmental conditions such as alveolar bone dehiscence are often associated with gingival recession.

Gingival recession may cause dentinal hypersensitivity, esthetic problems and could predispose to root decay. As far as hypersensitivity and esthetics are concerned, treatment is not mandatory unless the patient desires it. Dentinal hypersensitivity may be associated with recession caused by aggressive oral hygiene procedures and recession consequent to periodontal disease. [18] In the latter case, the condition is usually termed "root sensitivity", and may be a result of both periodontal disease per se and a consequence of periodontal surgical or non-surgical therapy. Non-surgical options are also available in the therapy of recession defects. Hypersensitivity should be treated, because it could prevent ideal oral hygiene measures and lead to plaque accumulation and further plaque-induced gingival recession. Esthetic concerns caused by gingival recession may be also be treated non-surgically by using an array of restorative techniques. The surgical treatment of recession may be accomplished through a variety of techniques and using different materials.

According to the literature, because it results in an exposed root surface, recession could furthermore be associated with an increased prevalence of root caries. Cementum and dentin are less resistant to the acidic and bacterial challenges inside the oral cavity and therefore may more easily develop carious lesions. Bignozzi et al. [19] have found an odds ratio of 2.4 for severe loss of attachment and an odds ratio of 1.05 for exposed root surface in the development of radicular caries. Prevalence of root caries can reach up to 90% higher in subjects with gingival recession compared to the rate in matched recession-free subjects, (20-40%). [20] The occurrence of non-carious cervical lesions may also be strongly associated with gingival recession, as determined in a cross-sectional study. [21]

The etiopathogenesis of recession was described in a classic article by Baker and Seymour. [22] The authors studied the mechanism by which recession was produced in an animal model, by inserting acrylic material into fresh extraction sockets. The paper confirmed the mechanism proposed in previous studies [23, 24, 25] which had identified the causal agent of recession as a localized inflammatory process. According to this theory, the local inflammation would induce proliferation of the pocket and oral epithelia, which would therefore begin to approach each other. Concurrently, destructive enzymes released by cells of the inflammatory infiltrate, (epithelial cells, fibroblasts, neutrophils and macrophages), would degrade the connective tissue component causing it to progressively thin out. The rete pegs of opposite epithelia would start to elongate and a connecting chord of epithelium would then form between the two surfaces. The cells derived from the oppositely polarized basal layers would then begin to progress towards maturity and begin to keratinize. As the two opposing surfaces begin to separate, the clinical result would be a small cleft. Simultaneously within the epithelium, maturation of the granular layer would occur at a progressively deeper level, resulting in desquamation. This process of desquamation would not be counteracted by a corresponding proliferation of cells, therefore leading to recession. The authors indicated inflammation as the primary etiologic agent. This may appear to contrast with the observation that most subjects who develop recession actually have excellent oral hygiene and are overzealous toothbrushers. Vigorous toothbrushing could lead to an increase in epithelial permeability or a physical alteration of the gingival tissue, thereby increasing susceptibility to inflammation. Furthermore, the amount of inflammation necessary to induce gingival recession is minimal and often not visible clinically.

THERAPEUTIC OPTIONS FOR ROOT COVERAGE

Many different surgical techniques exist for the treatment of gingival recession, with comparable outcomes in terms of mean root coverage. The selection among surgical techniques depends on the specific case and on factors such as the number and size of recession defects to treat, the presence or absence of keratinized tissue apical or lateral to the defect, the presence and height of interdental papillae, the presence of muscular pull or frena and the depth of the vestibule.[10]

Surgical procedures for root coverage can be divided in two major categories, namely pedicle soft tissue grafts, (including coronally advanced flap, rotational flap procedures, and regenerative procedures), and free soft tissue grafts, (these include the epithelialized gingival graft and the subepithelial connective tissue graft).

The coronally advanced flap, (CAF), has been documented extensively in the literature and is associated with good treatment outcomes. The coronally advanced flap is based on the coronal shift of the soft tissues to cover the exposed root surface. This technique is associated with excellent esthetic results and is less painful for the patient because no graft tissue is harvested from the palate / tuberosity area. According to the first publications, [26]case selection is very important because an adequate amount of keratinized tissue must be present apical to the recession defect in order for the results to be predictable. Furthermore, variables such as flap thickness [27] and flap tension [28] may play a significant role in determining the amount of root coverage that may be achieved. The "classic" technique [26] involved a split-thickness flap with two vertical incisions and one intrasulcular incision and was associated with excellent clinical outcomes, (97% mean root coverage). Subsequently, modifications were introduced in order to improve the healing. For instance, Pini-Prato et al. [29] introduced divergent incisions in order to provide a wider base for the flap and suggested that the flap be raised full-thickness to provide a thicker tissue over the exposed root surface.

Similarly, Zucchelli [30] introduced a modification of the coronally advanced flap to address multiple adjacent recession defects in the esthetic zone. This approach involves an envelope flap with no vertical incisions and aims to provide a better tissue adaptation when the flap is coronally advanced, particularly in the interdental areas. The modification, made to avoid vertical releasing incisions, is advantageous because it preserves the maximum amount of blood supply possible and reduces the risk of unaesthetic white scars. The proposed flap elevation is "split-full-split", which maintains a greater flap thickness over the area of root exposure while ensuring optimal flap mobility. In their study, the authors report a mean root coverage of 97%, and complete root coverage was achieved in 88% of sites. Interestingly, the amount of keratinized tissue apical to the recession defect demonstrated an inverse relationship with the amount of root coverage achieved after 12 months, meaning that there was more root coverage when a minimal band of keratinized tissue was present at baseline.

The coronally advanced flap has been applied in conjunction with enamel matrix derivatives, (EMD), [31] for treatment of recession defects. The rationale of this treatment option lies in the possibility of achieving greater mean root coverage if enamel matrix proteins are associated with a coronally positioned flap. In this case, it would be possible to avoid harvesting connective tissue from the palate or the tuberosity areas [32]. The enamel matrix derivatives are generally applied to the root surface after root conditioning with

ETDA and the flap is coronally advanced over the treated root surface. Castellanos et al. [32] found a significant difference in vertical mean root coverage between the sites treated with CAF and EMD, (88.6%) versus sites treated with CAF alone, (62.2%). Furthermore, there was a significantly greater gain in keratinized tissue in the test group, (CAF+EMD). Similar results were reported by Pilloni et al. [31] On the other hand, Modica et al. [33] did not find any differences in their split-mouth study between patients treated with EMD+CAF and patients treated with CAF alone. One possible explanation for this discrepancy lies in the different follow-up time, since Modica et al. [33] observed the outcomes up to 6 months, while Pilloni et al. [31] extended their observation period to 18 months.

Traditionally, the coronally advanced flap was considered inappropriate when there was minimal or no keratinized tissue apical to the recession defect to be corrected. Hence, the subepithelial connective tissue graft was proposed in 1985 by Langer and Langer, [34] as a means to correct recession defects on multiple maxillary teeth. The authors explain that the connective tissue graft represents a combination between the free gingival graft, (which ensures good quality of connective tissue), and a pedicle flap which provides optimal blood supply. The technique entails a partial thickness flap in the recipient area which will promote an adequate blood supply to the graft from the most superficial part of the flap and the underlying connective tissue and periosteum. No attempt was made to cover the graft completely in the recipient area. The authors did not record average root coverage, although the reported increase in root coverage was claimed to be between 2 and 6 mm. From an esthetic point of view, the proposed connective tissue graft technique introduced an improvement in the appearance of the recipient site, since the keloid-like appearance typical of the free gingival graft was rarely noticed.

Harris proposed a partial-thickness double pedicle flap associated with a connective tissue graft and reported high values of mean root coverage and complete root coverage, (approximately 97% and 80% respectively), pleasing esthetic results to the patients as well as reduced post-operative dentinal sensitivity. [35]

Among the most commonly used modifications of the original subepithelial connective tissue graft technique proposed by Langer and Langer [34], one of the most commonly used was proposed by Bruno. [36]The recipient site is prepared with butt joint incisions at or coronal to the level of the CEJ, and the author didn't attempt to cover the graft completely but rather left it largely exposed. The recipient site was prepared so as to ensure the highest possible amount of vascular supply to the area, by avoiding vertical releasing incisions and by harvesting the connective tissue graft with the underlying periosteum. This approach may, according to the author, provide an additional blood supply to the graft and is important especially in those cases of wide areas of root exposure.

More recently, some modifications to the original technique have been proposed to improve esthetics and post-operative discomfort. A split-mouth study by Zucchelli et al. [37] investigated the differences between a "traditional" approach, (control group), in which the graft thickness was greater than or equal to 1 mm and the graft was positioned at the CEJ and the test group. In the test group all the harvested connective tissue grafts measured less than 1 mm in thickness and were positioned apical to the CEJ, at a distance corresponding to the height of keratinized tissue apical to the recession defect prior to the surgery. The two groups yielded non-statistically significant differences in terms of mean root coverage and complete root coverage, but better esthetic results and less post-operative discomfort were reported by patients in the test group. These findings suggest that thinner grafts positioned apically to the CEJ may yield better esthetic results.

One of the main disadvantages of using the subepithelial connective tissue graft is the limited amount of tissue that may be harvested from a given patient. This is particularly problematic when multiple or large recessions need to be treated. Furthermore, patient discomfort is gaining increasing attention from clinicians and there is great interest in developing alternative sources of graft material to improve patient acceptance of treatment.

The development of a connective tissue substitute is still in a very early phase of development. [38] Nonetheless, there are three main possible sources of graft substitutes, namely allogeneic, xenogeneic and alloplastic. There are currently no available alloplastic materials that could substitute the connective tissue graft. Among the allogeneic materials, acellular dermal materials are the most commonly used, and many studies report excellent results in terms of root coverage [38]. Other tissue engineered allogeneic materials have been recently developed, such as the use of fibroblasts cultivated on different scaffold materials[39].

Xenogeneic substitutes of connective tissue grafts include porcine-derived collagen matrices. These products were initially utilized for keratinized tissue augmentation, but have also been employed for root coverage procedures. Cardaropoli et al.[40] compared the use of a decellularized xenograft with coronally advanced flap to a coronally advanced flap alone. At 6 months there was no statistically significant difference in recession reduction between the two groups, although there was a significantly thicker gingival tissue in the test group. Another study by the same authors[41] compared a porcine-derived collagen matrix with a subepithelial connective tissue graft for root coverage in single recession defects. In this publication, the authors found no statistically significant differences at 12 months in mean root coverage between test and control groups, (94.32% versus 96.97% respectively). Other parameters such as keratinized tissue gain and gingival thickness were also comparable between groups.

Among the materials that have been gaining increasing interest as alternatives to autogenous tissue are resorbable collagen membranes, that are already used in guided tissue regeneration (GTR) for intrabony defects. The rationale for using these materials lies in their composition, because the collagen contained in the membrane may eventually be replaced by newly formed collagen fibers and thus increase tissue thickness, which is known to be a key factor in preventing the progression of gingival recession. A split-mouth study[42] with 6-month follow-up found no statistically significant differences in mean root coverage between a coronally advanced flap with autogenous connective tissue graft, (control), and coronally advanced flap with bovine-derived collagen membrane, (test), although the mean root coverage was always greater in the control group. Interestingly, the probing depth in the test group was significantly smaller than the control, suggesting that a different type of attachment may be formed with GTR compared to CTG. In conclusion, resorbable collagen membranes may be a valid alternative for the treatment of gingival recessions. However, studies with a longer follow-up time are necessary to establish the long-term outcomes of GTR for root coverage.

The development of a substitute material for autogenous connective tissue is still in its infancy. The optimal material would ideally possess tissue-genetic and tissue-inductive properties, thanks to tissue engineering technologies. The ideal material would present minimal or no shrinkage, re-vascularize quickly and heal by secondary intention. As the properties of autogenous connective tissue vary depending on where it is harvested, realistically more than one substitute of autogenous tissue will be developed, each one with specific properties and indications of use.

PERIODONTAL BIOTYPE

The concept of periodontal tissue biotype has been investigated by different authors. A growing body of evidence has underlined that particularly thin tissues may pose esthetic and functional challenges to the clinician. In particular, it has been shown that there may be a relationship between the thickness of the soft tissues and the thickness of the underlying bone [43], which could in turn impact the response to certain treatments.

Thick gingival biotype is commonly associated with the classic image of periodontal health. The tissue is dense and often fibrous in appearance and displays a large area of attachment. The gingival topography is oftentimes quite flat and has a less pronounced scalloped appearance compared to a thin biotype. This usually corresponds to a thick underlying osseous morphology. In contrast, a thin biotype appears delicate and almost translucent. Soft tissue topography displays an accentuated scalloped appearance, which suggests very thin bone over the labial aspect of the roots. The bony anatomy is furthermore often associated with dehiscences and fenestrations [44].

The morphological characteristics of the gingiva are related to a number of factors, for instance the profile of the underlying hard tissues, the tooth and root form and the position of the teeth. The anatomy of the gingiva appears to be related to the contour of the osseous crest, as indicated by Becker et al. [45] These authors classified dry skulls into 3 different categories, namely flat, scalloped and pronounced scalloped. They observed that the pronounced scalloped category tended to be associated with longer teeth and a longer height of interdental bone. The authors concluded that there is a definitive relationship of the profile of the hard tissues and the length of the interdental bone, with the length and thickness of the interdental papilla.

Olsson and Lindhe [46] investigated the association between subjects who had narrow or wide crown forms of the upper central incisors. They calculated the CW/CL ratio, (crown width to crown length ratio), in all subjects and divided the study population into two groups, with wide and narrow crown form respectively. The values of probing depth and recession were calculated for both groups. The results of the study showed that a smaller CW/CL ratio, (meaning a longer clinical crown), was associated with higher probing attachment level and recession values. Even when taking age into account, the subjects in the narrow group had experienced a higher degree of recession on the buccal surfaces compared to the wide crown group. This observation seems to prove that the crown form is associated with the concept of periodontal biotype, and that subjects with thinner biotype will typically exhibit longer and more tapered crowns. However, data must be analyzed with caution due to the cross-sectional nature of the study, which may not reflect the causes of the loss of attachment and recession observed and but the association between crown form and biotype. Other studies have found an association between the shape of crown of the maxillary central incisor and the height of the keratinized mucosa, the interproximal maxillary central papilla and the gingival thickness [47].

HOW BIOTYPE IS MEASURED:

The concept of periodontal biotype is currently of great interest especially with regards to the effect it may have on treatment outcomes. Studies investigating the importance of periodontal biotype have devised methods to measure it and therefore evaluate its impact. A method which allows measurement of gingival thickness in a precise and predictable way is therefore crucial in order to draw appropriate conclusions.

De Rouck et al. [48] have proposed the use of a periodontal probe to distinguish thin from thick gingiva. While probing the buccal sulcus, the transparency of the probe can be observed through the gingival margin. The results of the study showed that there was a high intra-examiner reproducibility of gingival thickness measurement when using this method. Correlation of the measurements made by this method with clinical parameters such as probing depth, papilla height, gingival width and crown width/length ratio pointed to three clusters corresponding approximately to the biotypes. Furthermore, this method appears more precise than the simple correlation between tooth form and gingival thickness, which had already been disproven in previous studies [46].

Muller et al. [49] investigated the masticatory thickness in subjects with different periodontal phenotypes using an ultrasonic device to measure the gingival thickness in different areas of the mouth. The device uses a piezo-crystal to produce ultrasonic pulses at a frequency of 5 MHz which travel through the gingival tissues. The pulses travel through the gingival tissues at a speed of 1518 m/s and are partly reflected when they encounter tooth structure or bone. The device allows 1000 signals to be transmitted, received and analyzed. The transducer probe has a diameter of 4 mm and should be applied to moist tissues. By calculating the time necessary for the echo to be reflected back, the mucosal thickness may be determined and displayed digitally with a resolution of 0.1 mm, (within 2-3 seconds) [49]. A second study conducted by the same authors [50] investigated the reproducibility of measuring gingival thickness using the same ultrasonic device. An overall reproducibility coefficient of 1.20 mm was found, with large variations between different teeth. In particular, there was a higher variability between teeth with thicker gingiva, namely the posterior teeth, whereas teeth with thinner gingival tissues yielded more reproducible measurements. The authors concluded that the device is unsuitable to detect micrometer variations in gingival thickness that occur in gingivitis.

Ayub et al., [51] have utilized an anesthesia syringe with a silicone stop. The needle's position was standardized by using an acrylic stent with an orifice. The needle was placed through the orifice, perpendicular to the gingival tissue and the silicone disc stop placed in close contact with the gingiva. The thickness was then calculated as the distance between the silicone stop and the end of the needle and measured using a digital caliper.

Other authors [52] have utilized a less precise means of determining gingival thickness, namely a UNC probe inserted perpendicularly into the gingival tissues 2 mm apically to the mucogingival junction until the probe reached the alveolar bone. The readings were rounded to the nearest 0.5 mm.

THE EFFECT OF BIOTYPE ON PERIODONTAL DISEASE:

The association between gingival thickness and periodontal parameters indicative of disease status has been investigated in numerous studies.

De Rouck et al. [48] identified three clusters, namely A1, (slender tooth form associated with transparent gingival tissues on both maxillary incisors), A2, (no difference in clinical parameters compared to A1 but displaying clear, thick gingival tissues) and B, (thick flat biotype, with quadratic tooth forms, short papillae and thick, clear gingival tissues which masked the appearance of the periodontal probe). These authors could find a statistically significant difference in probing depth (PD) between clusters A1 and B, displaying mean PD value of 1.23 and 1.55 respectively. Although these differences appeared minimal, they confirmed the results found previously by others [46].

The observation that thick and thin gingival biotype exhibit profoundly different responses to inflammation was also noted by Kao and Pasquinelli [44]. Thick tissue typically responds better to inflammation. Following acute inflammatory stimuli, the lesion is typically encapsulated with resulting abscess formation, while chronic inflammation generally leads to a change in the appearance of the tissues, which display marginal erythema and an edematous and bulbous appearance. Persistent inflammation often causes pocket formation, which may be associated with the formation of infrabony defects due to the bulk of the bony component. These infrabony osseous defects which result from inflammation are often associated with thick bone buccolingually and or with tori on the labial or lingual aspect. Thin tissue, on the contrary, generally responds to inflammatory stimuli by formation of clefts and recession. More specifically, acute inflammation not only leads to abscess formation, but also to gingival recession and cleft formation. As the inflammatory stimulus becomes chronic the thin periodontal biotype continues to lose attachment by gingival recession and in most cases is associated with minimal probing depths [44].

Baker and Seymour [14] investigated the relationship between gingival recession and thin biotype. Recession was more frequently encountered in thin tissues and according to the authors, this finding could be explained by the fact that in thin tissues even a minimal amount of inflammation could occupy a substantial fraction of the volume of the tissue. In contrast, when thick tissues are present, the inflammation can be contained in the sulcular region and not extend enough to cause recession. Thin bony septa, which are most often associated with thin gingival tissues, may also contribute to soft tissue recession, because they are more susceptible to the effect of inflammatory exudates spreading through the gingiva and mucosa. In the author’s opinion, occlusal trauma acting on a thin bone and transmitted through the periodontal ligament could also lead to its resorption and act as a contributing factor to gingival recession [53].

Possibly, occlusal trauma could precede gingival recession leading to bone resorption. This would lead to a periosteal-periodontal attachment and a weaker soft tissue component, which would easily allow the spread of inflammation.

BIOTYPE AND DENTAL IMPLANTOLOGY:

Periodontal biotype has been associated with the outcome of various kinds of periodontal and surgical treatments. Implant treatment planning requires special attention in thin periodontal biotypes. A thin biotype is typically associated with a thinner and more fragile underlying alveolar bone plate. For this reason, extractions must be conducted with special care in order not to cause excessive damage to the bony walls of the socket and ridge preservation is often recommended. Additionally, in cases with compromised residual bone plate, it is necessary to contemplate more advanced solutions such as guided bone regeneration and possibly grafting with block grafts. Furthermore, the timing of implant placement may change upon consideration of the periodontal biotype. For instance, immediate placement should generally be avoided in cases of thin tissue biotype because the tissues may not be thick enough to mask underlying bone resorption, leading to esthetic concerns. In addition, the thickness of the bone may not be sufficient to guarantee osseointegration. The limited thickness of the tissue may also favor a greyish appearance of gingiva overlying the implant, particularly if the buccal bone is thin [53].

Romeo et al. [54] studied the effect of various parameters on the esthetic outcome of immediately placed single implants, and more specifically the presence of papilla. The results of the study indicate that thick biotype, as well as an implant-tooth distance between 2.5 and 4 mm and a contact-point to bone distance of < 7 mm, predictably resulted in the presence of a papilla. On the contrary, a thin tissue biotype was not significantly associated with papilla formation between tooth and implant.

BIOTYPE AND PERIODONTAL PLASTIC SURGERY:

A thin gingival biotype may yield a less positive outcome to treatment, as has been investigated in numerous papers [44], [55]**.** This is possibly due to the reduced vascularity inherent with thin soft tissue biotype, which could have a negative impact on wound healing and increase the risk of gingival recession [52]. In a study using fluorescein angiography, Mörman and Ciancio [56] analyzed vascular changes in the human gingiva following various kinds of periodontal procedures. The authors showed that the survival of split-thickness flaps depends upon the quality of the recipient vascular bed. Partial thickness flaps or flaps designed to cover an avascular area should not be too thin, because this increases the risk of necrosis due to insufficient vascularity. This finding suggests that thin flaps are at greater risk of post-operatory complications such as necrosis due to the lack of blood supply, which is crucial during healing. Clodius et al. [57] investigated the survival of thin or thick pedicle flaps separated from their bed and found that thick pedicle flaps had a survival rate of 55.7%, while thin pedicle flaps had a survival of 26.5%. Root coverage procedures are particularly delicate in relation to tissue thickness, because survival of the grafted tissue on the avascular root surface is critical to achieve success. The specific re-vascularization process will depend on the particular surgery being performed, but in general adequate vascularization of the flap and recipient bed is crucial. Berlucchi et al. [58] investigated the effect of anatomical factors on root coverage of Miller class I and II gingival recession defects treated with a coronally advanced flap. The authors divided the data among two groups, defects with a baseline recession height of < 4 mm and baseline recession height ≥ 4 mm respectively. Gingival thickness was measured 3 mm below the free gingival margin. Recession height ≥ 4 mm was associated with a decreased percentage of root coverage, (85.8% versus 91.4% in recession defects < 4 mm in height). The distance between the CEJ and the bone crest was negatively correlated with the amount of root coverage in both groups. Papilla width was weakly correlated with root coverage, but not papilla height. However, the study could not determine the nature of the relationship between papilla width and root coverage. The authors also found a correlation between the amount of root coverage and the thickness of the flap at baseline. Interestingly, when the data are grouped according to baseline recession height, there appeared to be a difference in root coverage in defects with initial recession height < 4 mm and in ≥ 4 mm. More specifically, complete root coverage was achieved in the group with deeper baseline recession defects only when the flap thickness was ≥ 1 mm, while flap thickness was less critical in shallower recession defects. The authors' proposal is that in deeper defects there may be more tension, which could potentially compromise blood supply to the flap margin.

A systematic review by Hwang et al. [52] investigated the effect of flap thickness as a predictor of root coverage in different mucogingival procedures. Despite the heterogeneity in treatments investigated and measurements of the gingival thickness in the included studies, the authors reported that thin flaps were negatively correlated with root coverage.

Due to the lack of standardization, the study was unable to yield a conclusive value for a minimum tissue thickness.

Baldi et al. [1] investigated the effect of flap thickness in determining root coverage in coronally advanced flaps. The authors reported that flap thickness was significantly associated with root coverage in shallow recessions. A thickness of 0.8 mm or higher was associated with complete root coverage. Therefore 0.8 mm was considered to be a threshold value above which complete root coverage can be expected. Although Huang et al. [52] have proposed different values of gingival thickness as threshold values, the concept that a thicker biotype, and therefore a thicker flap, is associated with a greater degree of root coverage, was confirmed. In this particular study, the authors reported that gingival thickness at baseline measured with bone sounding was associated with complete root coverage if greater than or equal to 1.2 mm.

Other factors contributed to degree of root coverage, such as the location of the tooth in the mouth and the age of the patient, with mandibular teeth and a greater age yielding less root coverage and recession depth reduction. The difference in gingival thickness reported compared to the previous study may have been due to the method of thickness measurement and the level at which the measurement was recorded. In the study by Baldi et al., [1] measurements were made at the midpoint between the mucogingival junction and the flap base, meaning the horizontal distance between the most apical extent of the vertical releasing incisions. In contrast, Huang et al. [52] measured the tissue thickness 2 mm apically to the gingival margin using by penetrating a UNC probe into the soft tissue and rounding off to the nearest 0.5 mm.

ACELLULAR DERMAL MATRIX: DEFINITION

Acellular dermal matrix, (ADM), is an allograft material that has been chemically processed in order to remove all cellular components, so that only the connective tissue matrix remains. ADM is an allograft of human skin that has been freeze-dried or lyophilized. All cells in the epithelial and connective tissue compartments are removed, and the resulting product is a de-cellularized connective tissue graft with an underlying basal lamina. The extracellular matrix, including the vascular channels, remains intact. [59]

Removal of cellular components increases the safety of the material, because these are necessary for survival and transmission of viruses [60]. ADM functions as a scaffold and provides collagen, elastin, blood vessel channels and proteins. ADM heals by cell repopulation and revascularization through preserved vascular channels. In order for revascularization to occur, ADM must receive as much blood supply as possible when placed on an avascular root surface, as is the case in root coverage procedures. Tunneling and other flap designs conceived to preserve vascular integrity have been developed for this purpose [61].

In comparison with an autogenous connective tissue graft, ADM does not require harvesting from the palate and does not imply a second surgical site. Therefore, ADM is particularly advantageous in large or multiple recession defects or in patients who have very thin palatal tissues. It may also be a valid choice for those patients who do not desire the morbidity associated with a second surgical site. [61]

HISTORY

ADM may be used in the medical field for the treatment of extensive burns in dermatology. This material is mainly used as an effective temporary dressing, because generally it will not take to a full-thickness skin wound. Instead, it will often be rejected in the long-term and slough off. Among its applications, ADM has also been proposed in cosmetic surgical procedures, for instance in lip augmentation surgery.

The first application of ADM in periodontal surgery was in mucogingival procedures and was more specifically designed to increase keratinized tissue in free gingival graft procedures [62].

CHEMICAL AND PHYSICAL CHARACTERISTICS

A histological study by Scarano et al. [63] investigated the application of acellular dermal matrix to gingival augmentation procedures. The authors observed a clinical gain of keratinized tissue of 2.9+/- 0.65 mm after 3 months. Ultrastructurally, the authors noted macrophages phagocytosing pre-existing collagen fibers in the first few weeks. Starting from the second week, the authors noted fibroblasts synthesizing new collagen fibers, epithelial cells colonizing the graft surface and re-vascularization. The process was terminated at 10 weeks, when the epithelialization was completed and a well-structured basement membrane was present.

Cummings et al. [64] investigated the histological aspects of healing with acellular dermal matrix after a 6-month healing period. The ADM site was compared with an autogenous connective tissue graft, and both mucogingival procedures were performed on teeth that were scheduled for extraction. The histological analysis of sections treated with ADM revealed a complete incorporation of the graft and an increased thickness of the connective tissue in correspondence of the grafted areas. The underlying collagen fibers of the host tissues had a similar density and histologic appearance compared to those of the overlying area corresponding to the graft placement. Both the samples treated with autogenous connective tissue and those treated with ADM revealed an increased thickness in the buccolingual direction. The ADM also had an abundance of elastin fibers, which allowed it to be distinguished from the alveolar mucosa. Although the mucosa is naturally rich in elastin, it still had less elastin compared to the ADM grafted area. This confirms that acellular dermal matrix, when compared with gingival tissues, has a higher elastin content, which allows it to be easily identified microscopically. A notch was placed on the study teeth, and a minimal amount of osseous and cemental deposition was observed in both CT and ADM-treated teeth, although there was a high degree of inter-patient variability. At 6 months, the histological appearance of the graft confirmed that it was fully integrated within the gingival tissues and fibroblasts and endothelial cells were present, demonstrating re-vascularization. The authors also specify that the amount of root coverage obtained clinically was somewhat low, although the results are not reported. This may be explained by the poor oral hygiene and compliance of the patients involved, whose teeth were deemed hopeless and scheduled for extraction. Most defects were classified as Miller III or IV, and all patients smoked one pack of cigarettes per day. These considerations may be important when evaluating the healing and clinical applicability of the results.

USE IN DENTISTRY

The use of ADM is not restricted to mucogingival procedures and the material has many clinical applications. Novaes Jr. and Souza [60] have used ADM as a membrane for ridge preservation in a case report. In this study, the authors used no grafting material, claiming that the fenestration in the buccal bone was of insufficient size to cause the membrane to collapse within the defect. The authors decided to leave the membrane exposed to increase the amount of keratinized tissue and despite this decision, there were no signs of infection or membrane contamination at 4 weeks. After 6 months healing was complete and there was a gain in keratinized tissue on the area. The apparent resistance of ADM to bacterial contamination represents a significant advantage of this material, especially in those cases in which primary closure is impossible to achieve.

USE IN MUCOGINGIVAL THERAPY

Use of ADM in root coverage procedures has been validated by numerous studies that are presented in the following paragraphs.

Single recession

Novaes et al. [60] have evaluated the use of ADM with coronally advanced flap compared to subepithelial connective tissue grafts for root coverage in single Miller class I and II recession defects. The authors reported 2.10 mm of mean recession reduction for ADM versus 1.83 mm for subepithelial connective tissue graft and mean root coverage of 66.5% for ADM versus 64.9% for subepithelial connective tissue graft.

Multiple recessions

One of the main indications of ADM is the treatment of multiple gingival recession defects, where using autogenous tissue would imply harvesting a very large graft. [65]

Thombre et al. [66] have investigated the use of acellular dermal matrix in multiple adjacent recessions compared to coronally advanced flap with a 6-month follow-up. Treatment with ADM plus coronally positioned flap, (CAF), yielded a higher percentage of defects with complete root coverage, (63% compared to 24%), compared to CAF alone. Furthermore, the mean root coverage was significantly higher in the ADM group, (90% versus 66%) and the root coverage was more predictable with ADM+CAF compared to CAF alone. The authors proposed that the greater root coverage obtained with ADM might be due to the increased flap thickness resulting from its use. According to the authors, ADM is a predictable and convenient way of treating multiple recession defects and may improve the esthetic outcome by yielding a higher degree of root coverage compared to CAF.

Using a randomized clinical trial design, Ahmedbeyli et al.[67] compared the use of ADM plus CAF, (test), versus CAF alone, (control), alone in the treatment of multiple Miller class I gingival recessions in thin gingival biotypes with initial tissue thickness was < 0.8 mm for all patients enrolled. After 12 months, a statistically significant difference in favor of the test group was found for mean recession reduction, with values of 3.08± 0.51 for the test group and 2.37 ± 0.83 for the control group. Complete root coverage was obtained in 75% of the test defects but only 50% of the control defects. The sites treated with ADM plus CAF also showed a significant increase in gingival thickness at the 12-month time-point, (0.69 mm versus 0.07 mm in the control group), the authors interpreted this as significant finding of the study. Higher values of post-operative gingival thickness were, in fact, found to be associated with a higher percentage of complete root coverage.

Mucogingival surgery aiming at treating multiple adjacent gingival recessions is considered to be more challenging than the treatment of single recession defects. The larger avascular root surface area, the difference in recession height between adjacent teeth, the reduced blood supply and the difference in the position of adjacent teeth all contribute to the greater treatment challenge.

A recent systematic review by Hoffmänner et al.[68] analyzed outcomes of different treatment options reported in the literature for the treatment of multiple adjacent gingival recessions, (MAGR). The results of the review seem to indicate that the modified coronally advanced flap, (MCAF) results in complete root coverage over a 5-year period. The use of a connective tissue graft as an adjunct further increases the long-term outcome of MCAF. In terms of complete root coverage, the authors suggest that autogenous connective tissue grafts associated with various flap designs yield better and more predictable results compared to the use of bioabsorbable materials such as ADM and PRF. These results should however be interpreted with caution, because most of the evidence is represented by low evidence case series and very few RCTs are available on the subject.

Due to its avascular nature, ADM relies on the blood vessels of the recipient site for its survival and revascularization. For this reason, it is important for the ADM to be placed by a surgical procedure that ensures maximum vascularization. In a split-mouth randomized controlled trial, Barros et al. [27] used ADM to treat 32 Miller class I and II defects. Two different surgical procedures were compared, namely the conventional flap design proposed by Langer and Langer [34] for the subepithelial connective tissue graft, (control group), versus a modified flap design, (test group). This modification involved displacing the vertical releasing incisions to the line angles of the adjacent teeth, thus providing a broader flap. At 6 months the authors reported statistically significant increases in root coverage for the test group (79%) versus 63.9% for the control group and concurrent mean recession reduction of 3.64 ± 0.64 mm for the test group versus 2.16± 0.97 mm for the control group. The more favorable results achieved with the modified flap design were attributed to the potentially improved vascularization of the flap, which would provide more cells and nutrients necessary for incorporation of the ADM allograft, although this cannot be confirmed because no histological analysis was conducted.

A case series by Mordaressi and Wang [69] evaluated the use of ADM with a tunneling technique and showed that the use of ADM could predictably achieve success in root coverage procedures for the treatment of multiple adjacent recession defects. The mean root coverage achieved was 93.5% at 6 months, while an increase in gingival thickness of 0.15 mm was obtained 12 months post-operatively compared to baseline values.

Incision and flap design have not been extensively studied when applied to ADM, however some studies have compared a coronally advanced flap to a coronally positioned tunnel. For instance, Papageorgakopoulos et al. [70] found that, when treating single Miller I or II recession defects the coronally positioned flap yielded better and more predictable results than the coronally positioned tunnel. The percentage of root coverage achieved with the tunnel was 78% versus 95% achieved with the flap approach. Even more relevant was the predictability of obtaining root coverage > 90%, which was only 50% of sites for the tunnel versus 83% of sites using the flap. Additionally, at 4 months the tunnel group experienced a loss of root coverage while the control group had stable gingival margin levels. Gingival thickness measurements were also made with an ultrasonic instrument, at 2 and 4 months. The test group, (tunnel), only showed a 0.1 mm increase versus 0.5 for the control group. The authors concluded that the predictability of root coverage using a tunnel and ADM was inadequate.

LONG-TERM EVIDENCE

Long-term evidence on the use of acellular dermal matrix as a substitute for autogenous connective tissue is scarce. However, the available publications suggest there may be significant changes over long-term periods of time in the material's characteristics and stability. A two-year split-mouth study [71] with a two-year follow-up published in 2006 supports this observation. The authors measured gingival tissue thickness, keratinized tissue height, recession height and width, CAL and PD. Regarding the gingival thickness, recorded at the mid-point between the mucogingival junction and the free gingival margin, the greatest thickness was measured at the 6-month time-point. After this time, the material's thickness decreased consistently at 12 and 24-months.

A 2004 study by Harris [72] sheds some light on the long-term stability of sites treated with acellular dermal matrix. The author's findings suggest that ADM is significantly less stable compared to autogenous tissue grafts, as demonstrated in 25 patients treated with each modality. The long-term follow-up was conducted on average 48.2 months in the ADM, although this time-point differed between patients. The change in percentage of root coverage for the ADM, (93.4 to 65.8%), decreased substantially in comparison with a stable result in the autogenous group, (96.6 to 97%). There was a similar decrease in the rate of defects reaching complete root coverage, with a decrease of approximately 50% in the ADM group while there was an increase of such defects, (82.1 to 89.7%) in the autogenous group. From a methodological standpoint, this study presents serious flaws, such as lack of randomization of the intervention between groups, inclusion of single and multiple recession defects, no information regarding Miller classification of defects, different surgical techniques are among some of its shortcomings, etc., which could ultimately affect the validity of the scientific evidence presented. For all these reasons, the information presented in the study is of limited value and more studies with a long-term follow-up are desirable.

Long-term results with acellular dermal matrix have been compared to those achieved with autogenous connective tissue grafts for up to 5 years [71]. The study had a split-mouth design and included 16 patients, each with bilateral Miller class I or II single recession defects. The treatment intervention was randomly assigned to receive autogenous tissue or an allograft. Interestingly, the 6-month data showed better results with acellular dermal matrix compared to autogenous tissue, (73.3% of sites receiving CRC versus only 20% of sites in the autogenous tissue group; 85.42% mean root coverage for ADM versus 69.05% for the autogenous group). In the discussion section, the authors explain their data by admitting the inclusion of some Miller III recession defects in the autogenous connective tissue group, although this was not explained in the inclusion criteria section. This could potentially have a profound influence on the results.

At 60 months, on the other hand, the data showed a relapse with recurrent recession for both groups, with no statistically significant difference between the groups, (33% recurrence in the ADM group versus 27% in the autogenous group). The data on keratinized tissue width is also interesting. A similar increase was found at 6-months in both groups, however in the autogenous group values remained constant at 5 years, while the ADM group experienced a significant reduction in KTW. At 60 months, the KTW in the ADM group reached baseline values.

Despite some interesting observations, this study presents some serious shortcomings which should be kept in mind when interpreting results. For instance, measurements were made by a non-calibrated examiner with no stent and not using a digital caliper, but a periodontal probe and rounding off the results to the closest 0.5 mm. Also, no measurement of gingival thickness was recorded, which may contribute to explain long-term stability of the gingival margin levels.

Aim and Hypothesis

Aim: the present investigation aims to evaluate and compare the percentages of root coverage using ADM as the grafting material under a coronally advanced flap in Miller's class I and II multiple recession defects with either thin or thick periodontal biotype.

Hypothesis: it is hypothesized that there will be a difference in the mean percent root coverage obtained using ADM as a grafting material for the treatment of multiple recession defects. More specifically, a higher outcome in terms of percent root coverage is expected with a thick tissue biotype compared to a thin biotype.

Primary outcome: mean root coverage, expressed in percentage as the average of the two individual defect coverage values. The percent root coverage was expressed in two different ways: one was capped at 100% while the other was calculated as the arithmetic mean of the two individual root coverage values and was allowed to exceed 100%.

Secondary outcome variables: changes in clinical probing depth (CPD), clinical attachment level (CAL), recession height (RH), recession width (RW), keratinized tissue width (KTW) and gingival thickness (GT); complete root coverage (CRC), patient satisfaction, incidence of complications.

Research Design

Overview: Using a parallel study design, root coverage procedures were carried out in subjects with both thin and thick soft tissue biotypes. Both groups were treated with the same intervention, (i.e. coronally advanced flap with acellular dermal matrix graft). The outcomes were compared between the two groups at 3 months post-surgery.

The study protocol was reviewed and approved by the Scientific Review Committee, (SRC), and by the Institutional Review Board, (IRB). IRB approval was received in July 2015 and patient recruitment commenced soon after.

Materials and Methods

Inclusion criteria:

- Two Miller class I or class II adjacent recessions with a recession height≥2 mm and<4 mm; [73]
- In case there were more than two adjacent recession defects, only two were included in the study. The inclusion of the specific defects was determined randomly via the "sample" function of the statistical software package R (Version 2.13.1);
- Systemically healthy subjects with no contraindications to mucogingival surgery
- Anterior teeth: incisors, canines or premolars;
- Patients having full-mouth PI and GI<1, calculated as the average value of PI and GI for the patient.

Exclusion criteria:

- Molar teeth;
- Subjects taking medication known to interfere with gingival metabolism and cause soft tissue enlargement (cyclosporine A, calcium channel blockers, phenytoin)
- Subjects suffering from systemic conditions which influence wound healing: uncontrolled diabetes, (HbA1c>6.5%), connective tissue disorders such as Ehlers-Dahnlos syndrome, subjects taking immunosuppressant medications (corticosteroids or cytostatics), immunosuppressed subjects suffering from AIDS or other conditions causing immunosuppression;
- Pregnant or lactating subjects;
- Teeth with large restorations whose margins impinge on the CEJ or make identification of the CEJ impossible;
- Teeth with severe occlusal interferences;
- Teeth with deep cervical lesions, (horizontal distance between the projected tooth convexity and the deepest part of the cervical lesion≥2 mm);
- Areas previously treated with mucogingival surgeries.

All subjects were enrolled in the study only if they have a full-mouth gingival index (GI) and plaque index (PI) less than 1. Whenever indicated, phase I therapy (namely oral hygiene instructions, ScRP, prophylaxis, removal of plaque- and calculus-retentive factors and occlusal adjustments if necessary) was performed prior to the enrollment. Patients who qualified to enter the study were instructed to use a roll-technique to eliminate habits possibly related with the etiology of the recession defect. At the same appointment, baseline gingival thickness (GT) and other clinical parameters were assessed.

All measurements with the exception of GT were taken and recorded by one examiner (LPH). GT at baseline and at all subsequent visits were measured by the PI, (WSC). This was done in order to ensure that the surgeon was blinded as to which group the subjects belonged and therefore helped to reduce bias.

The included subjects were divided in two groups according to gingival thickness: Thin gingival biotype group (TnB)<0.8 mm, thick gingival biotype group (TkB)≥0.8 mm. Each subject was in one of the two groups only. In the present study, gingival biotype was defined as gingival thickness and only the dimension of the soft tissue was considered, as opposed to measuring or observing the underlying bone upon flap elevation.

The examiner was calibrated to the Principal Advisor (WSC). In addition, intra-examiner calibration was carried out by repeating the clinical parameters (RH, RW and KT height) at least 48 hours after the first set of measurements. The calibrations were carried out on fellow residents. The two sets of measurements were compared to determine intra-examiner reproducibility.

Custom-made acrylic stents were fabricated and used for each patient in order to ensure reproducible measurements and reduce potential errors related to incorrect probe placement. A digital caliper with 0.05 mm resolution (Mitutoyo Advanced Onsite Sensor Absolute Scale Digital Caliper, 0-6") was used to take the measurements. These parameters were measured at baseline, 3 months post-op. See figure 1 for more details.

The clinical parameters were defined, measured and recorded on an Excel spread sheet (referring to Form A in the Appendix) as follows:

- PI (plaque index), GI, (gingival index) [75] and BOP (bleeding on probing) [76]. PI and GI of the single experimental site will be calculated at each study visit;

- CAL (clinical attachment level): the distance between the CEJ and the base of the pocket);
- PD (probing depth): the distance between the free gingival margin and the base of the pocket);
- RH (recession height): the distance between the CEJ and the free gingival margin at the most apical point of the recession margin;
- RW (recession width): the horizontal distance from one border of the recession to the opposite border of the recession at the level of the CEJ;
- KTW (keratinized tissue width): the distance between the free-gingival margin and the muco-gingival junction;
- GT (gingival thickness): the thickness of the alveolar mucosa 1 mm apical to the free gingival margin. After having numbed the area with topical anesthetic, a 32G anesthetic needle with a silicon rubber stop was placed perpendicular to the mucosa until a hard surface is felt. The distance between the tip of the instrument and the silicone disc stop was then measured.

For recession defects associated with an unidentifiable CEJ, the contralateral homologous tooth was employed.[74] More specifically, two periodontal probes were used on the contralateral tooth to identify reference points that were then transferred to the tooth in question. The first probe was positioned horizontally across the CEJ at the base of the interdental papillae, while the second probe was placed along the long axis at the center of the tooth. The most mesial- and distal-coronal points of the CEJ and the intersection between the CEJ and the long axis of the tooth were identified. The mesiodistal width of the anatomical crown at the base of the interdental papilla and the distance between the intersection of the CEJ with the long axis of the tooth and the incisal margin were measured. Using the two periodontal probes in the same position, the measurements and reference points were transferred to the test tooth. For cases in which the contralateral tooth did not have recession, the distance between the incisal margin and the intersection between the vertical probe and the CEJ was measured, added to the probing depth. Finally, if the CEJ was not identifiable on the contralateral tooth either, the CEJ of the adjacent tooth or teeth were used as a reference.

Furthermore, satisfaction surveys (Form B in Appendix, Mahajan et al. [77], 2007) were passed out to the enrolled patients. The patients were questioned on root coverage obtained, relief from hypersensitivity, color shape and texture of gums, post-surgical pain, swelling and discomfort, and post-operative complications.

Clinical photographs of a fixed magnification were taken throughout the course of the study. In order to make the photographs as reproducible as possible, the same F stop and exposure time were used consistently. Photographs were taken with the same camera and lens at all times, (Nikon D5100 DSLR camera body; Nikkor lens, 105 mm, Nikon). The photographer angled the camera perpendicularly to the site to the best of her abilities.

Surgical Technique:

The surgical technique was a modified coronally advanced flap with no releasing incisions. As previously demonstrated, vertical releasing incisions cause an interruption in vascular supply. The coronally advanced flap with no releasing incisions yields a greater amount of buccal keratinized tissue height, a reduced post-operative discomfort and a better esthetic result with less keloid formation compared to a coronally advanced flap with vertical releasing incisions. [30] Furthermore, the absence of vertical releasing incisions may represent an advantage when using ADM, because it may decrease the chance of graft exposure. [30]

All surgeries were performed by a single operator (LPH). The incision design was the one proposed by Zucchelli and De Santis, [30] which avoids releasing incisions ensuring maximum blood supply. A full-thickness mucoperiosteal flap was extended 1 mm apical to the MGJ and a split-thickness dissection was performed mesially, distally and apically in order to allow adequate coronal positioning of the flap. The part of the root surface exposed to the oral cavity was planed thoroughly to remove all debris, plaque and calculus. The anatomical papillae were exposed and de-epithelialized to ensure the correct exposure of the connective tissue vascular bed. After the flap had been elevated the exposed root surface was conditioned with EDTA for 2 minutes.

The ADM was used according to the manufacturer’s guidelines and positioned at least 3 mm beyond the alveolar bone margin apically and 1 mm apical to the CEJ apico-coronally. Whenever possible, the apico-coronal height of the ADM was maintained constant at 8 mm, while mesio-distal length of the graft was dictated by the extent of exposed surfaces which needed to be covered.

The flap was then coronally positioned and secured with a continuous sling suture technique using a 5-0 p-3 poliglecaprone suture material. Periodontal dressing material was applied to the lingual surfaces of the involved teeth in order to isolate the knots and avoid interferences from the tongue.

Postsurgical instructions were given. Antibiotics, non-steroidal anti-inflammatory drug and a chlorhexidine mouthwash were prescribed. Patients were seen at 1 week, 3 weeks, 1 month, 3, 6 and 12 months.

Amoxicillin 500 mg three times daily for 7 days was prescribed. Clyndamycin 300 mg three times daily for 7 days was used in case of patients allergic to penicillin.

Statistical Analysis

Power calculation

A power calculation was conducted using nQuery Advisor (Version 7.0). The assumed values for percentage root coverage in the two groups (thin biotype and thick biotype) came from Figure 6 of Baldi et al.[27] Under the aforementioned definitions of the two groups (<0.8mm for the thin biotype group, and ≥ 0.8mm for the thick biotype group), and collapsing the results of Baldi et al.[27] into these two groups, the assumed mean (SD) for the thin biotype group was 70.17 (10.80); the assumed mean (SD) for the thick biotype group was 98.60 (3.96). Based on these values, a sample size of n = 10 per group is adequate to obtain a Type I error rate of 0.05 and a power greater than 99% for the independent-samples t-test comparing the two groups in terms of percentage root coverage. To account for possible attrition, an initial sample size of n = 13 per group was taken. The results of Hodges and Lehmann[78] indicate that if the Mann-Whitney U test is used instead of the independent-samples t-test, a power greater than 80% will still be obtained.

Statistical analysis

Descriptive statistics were computed by group. The assumption of normality was evaluated via quantile-quantile plots. For analyses in which the assumption of normality was found to be tenable, statistical significance was assessed using parametric methods (the independent-samples t-test for unpaired analyses, and the paired t-test for paired analyses); means and standard deviations were reported. If there was evidence that the assumption of normality was violated, statistical significance was assessed using nonparametric methods (the Mann-Whitney U test for unpaired analyses, and the Wilcoxon signed-rank test for paired analyses); medians and inter-quartile ranges were reported. Descriptive statistics are presented as mean ± SD unless otherwise specified. To assess associations between binary variables, the chi-square test was used (or Fisher's exact test in the case of sparse expected cell counts)., *P*-values less than 0.05 were considered statistically significant. SPSS Version 22 was used in the analysis.

Primary Outcome:

- The primary outcome, percent root coverage three months post-surgery, was calculated as the average between the two test teeth and expressed in two ways:
    - "mean percent root coverage" was obtained by simply averaging the root coverage achieved after three months for tooth 1 and tooth 2;
    - "capped mean percent root coverage" was obtained by setting the maximum percent root coverage the procedure could achieve on a test tooth as 100%. That is, if the value exceeded 100% for a given tooth, it would automatically be made to equal 100%. The capped average was calculated again as the average of the root coverage of the two teeth involved.

Complete Root Coverage: The defects that achieved complete root coverage, (root coverage 100%) were compared between groups. A pair of defects was considered to have achieved CRC when at least one of the defects had achieved complete root coverage.

The baseline VAS scores were compared between groups, and the changes between groups at three weeks and three months were also compared between groups. The difference between groups was calculated as follows: (first time-point VAS)- (second time-point VAS). Positive changes indicate that sensitivity is improving/ was alleviated after the surgery. Negative changes indicate that sensitivity is becoming more severe.

Results

Demographics:

A total of 26 patients were preliminarily recruited for the study. During the course of the study, 5 subjects dropped out for various reasons. It was not possible to identify them by group, however, because all of them left the study between visits 1 and 2, before GT measurements were taken.

The present study was conducted with a sample size of 21 patients, 11 of which belonged to the thin group, (11/21 or 52.4%) and 10 to the thick group, (10/21 or 47.6%). (Table 1).
Of the 21 included patients, 12 were males, (12/21 or 57.1% of the sample), and 9 were females, (9/21 or 42.9% of the sample). In the thin biotype group, 5 subjects were females and 6 subjects were males, respectively 45.5% and 54.5% of the total of the group. In the thick biotype group, 4/10 or 40% of the sample were females and 6/10 or 60% of the sample were males. The difference between groups was not significant, (p = 0.801).

The age of the total sample was 41.6±15.3. The age in the thin biotype group was 46.4±15.07 the average age in the thick biotype group was 36.4±14.3. There was no statistically significant difference for age among groups, (p=0.139). See table 1.

<u>Between Group Comparisons:</u>

Baseline parameters: among the baseline parameters, all were normally distributed except for GI, PI and RW. In this case, Mann-Whitney U test was used. All other differences were tested with the independent samples t-test.

GI: The average GI in the thick group was 0.24±0.22 whereas it was 0.16±0.14 in the thin group. This difference was not statistically significant, (p=0.426).

PI: The average PI in the thick group was 0.18±0.17, it was 0.28±0.25 in the thin group. These differences were not statistically significant, (p=0.282).

RH: In the thick group, the average recession at baseline was 2.63±0.57, while in the thin group it was 2.62±0.33. No significant differences could be detected, (p=0.920).

RW: The average recession width in the thick group was 3.40±0.57; in the thin group the average value was 0.36±0.74. The differences were insignificant, (p=0.557).

KTW: The mean width in the thick group was 2.69±0.92, while in the thin group the average value was 1.69±0.87. The comparison yielded statistically significant differences between groups, (p=0.018).

PD: Values were 2.11±0.72 and 1.62±0.58 in the thick and thin groups respectively. No statistical significance could be detected, (p=0.100).

CAL: Values were 4.64±0.97 and 4.48±0.74 respectively in the thick and thin groups. The difference among groups was insignificant, (p=0.666).

GT: The average in the thick group was 0.94±0.08 while it was 0.59±0.14 in the thin group. No statistical comparison was carried out between groups at baseline, because it was known that there would be a statistically significant difference between the groups by virtue of the way the groups were defined. Please refer to table 2, and figures 2, 3 and 6).

Three months:

At three months, all parameters were normally distributed except RW, therefore the Mann-Whitney U test was used. Note that negative numbers indicate that the recession defect is completely covered and that the free gingival margin lies coronal to the CEJ. See figures 4, 5 and 7 and table 2.

GI: The average in the thick group was 0.20±0.14 and 0.18±0.11 in the thin group. The differences were not significant (p=0.737).

PI: Respectively 0.37±0.28 and 0.39±0.23 in the thick and thin groups. The difference was not statistically significant, (p=0.818).

RH: Average in the thick group was -0.02±1.47 versus 0.18±1.18 in the thin group. The p value was 0.480, and the difference was not statistically significant.

RW: In the thick group the average was 0.59±1.26 versus 1.01±1.28 in the thin group. The difference was not significant, (p=0.557).

KTW: Respectively 2.49±0.96 and 1.58±0.62 were found in the thick and thin groups. The difference was statistically significant, (p=0.017).

PD: In the thick group, the average was 1.59±0.50 while in the thin group it was 1.64±0.45. The difference was not significant, (p=0.826).

CAL: The average values were 2.22±1.41 and 3.38±1.21 for the thick and thin groups respectively. The difference was not significant, (p=0.057).

GT: On average 1.58±0.20 was measured in the thick group while in the thin group the average was 1.21±0.30. The difference was significant, (p=0.004).

Primary Outcome at three months:

Mean percent root coverage: The independent samples t-test was used to compare the two groups at three months, (the data was normally distributed), (see table 3 and figure 8). The average root coverage in the thick group was 115.16±54.68%, while in the thin group it was 95.12±14.7%. The difference between the two groups was not statistically significant, (p=0.387).

Capped percent root coverage: The Mann-Whitney U-test was used to compare the two groups because the data was not normally distributed. Data are presented as median±IQR, (table 3, figure 9). The median root coverage in the thick group was 100.00±28.00 while in the thin group it was 92.36±62.80. The difference was not statistically significant, (p=0.282).

Complete Root Coverage, (CRC): Complete root coverage at three months was compared between groups using the Fisher's exact test. In the thin group, 7/11 or 63.6% of the subjects had complete root coverage, versus 8/10 or 80% in the thick group. The difference was not significant, (p=0.635). Refer to figure 10.

VAS score for Tooth Sensitivity

Baseline

The Mann-Whitney U test was used because the data was not normally distributed, no significant differences could be found among groups. The median sensitivity score was 5.00±4 and 3.00±5 in the thin and thick groups respectively. The difference was not significant, (p=0.847). See table 4. See figure 11.

Change in VAS scores for tooth sensitivity between groups at different time-points:

Negative changes indicate a worsening, while positive changes indicate an alleviation of sensitivity. The data was not normally distributed at any of the different time-points, therefore the Mann-Whitney U test was used.

Change between baseline and 3 weeks: In the thin group the median score was 1.00±4, while in the thick group it was 3.00±4. The difference was not significant, (p=0.193).

Change between baseline and 3 months: The median score in the thin group was 3.00±5, while in the thick group it was 2.00±3. No statistically significant differences could be detected, (p=0.562).

Change between 3 weeks and 3 months: In the thin group, the mean score was 0.00±4 while in the thick group it was 0.00±1. No statistically significant differences could be detected, (p=0.056). See table 4 and figures 12, 13, 14.

Rate of complications at different time-points.

The complication rate was assessed at different time-points and compared between groups. Complications were considered to be anything which deviated from normal healing, i.e. flap necrosis, graft exposure, wound dehiscence, excessive bleeding or swelling, infection of the wound. Complication was rated as either present or absent, and the Fisher's exact test was used to compare the frequency of complications between the two groups. See table 5 and figure 15.

Complication rate at 1 week: In the thick group, 4/10, (40%), subjects experienced some forms of complication at this time-point, while in the thin group 3/11, (27.3%) experienced complications. No statistically significant difference was detected, (p=0.659). Complications included excessive swelling with distortion of the face, graft exposure, excessive bleeding and flap necrosis.

Complication rate at 3 weeks: At this time-point 2/10, (20%) and 3/11, (27.3%) of subjects experienced complications in the thick and in the thin group respectively. There was no statistically significant difference, (p=1.000). The complications at three weeks included delayed healing as a result of flap necrosis and graft exposure.

Within-group comparisons:

Thick Biotype:

GI: At baseline the average was 0.34±0.22 while at three months it was 0.20±0.14. The difference was insignificant, (p=0.638).

PI: The average was 0.18±0.17 and 0.37±0.28 at baseline and three months respectively. The difference was not statistically significant, (p=0.063).

RH: The average at baseline was 2.63±0.57 versus -0.24±1.47 at three months. The difference between time-points was significant, (p<0.001).

RW: The median was 3.30±0.62 and 0.00±0.62 at baseline and three months respectively, and the difference was statistically significant, (p=0.001).

GT: The median was 0.92±0.16 versus 1.53±0.18 at baseline and three months respectively. The difference was not statistically significant, (p=0.101).

KTW: The average at baseline was 2.70±0.92 versus 2.49±0.96. The difference was not statistically significant, (p=0.485).

CAL: The average at baseline was 4.65±0.97 versus 2.20±1.41 at three months. The difference was statistically significant, (p=0.002).

PD: The average at baseline was 2.11±0.72, while at three months it was 1.59±0.50 with a non-statistically significant difference between groups, (p=0.101).

Thin Biotype:

GI: At baseline average value was 0.16±0.14 versus 0.18±0.11 at three months. The difference was not statistically significant, (p=0.722).

PI: Baseline and three month-averages were 0.28±0.25 and 0.40±0.23 respectively, with a non-statistically significant difference between groups.

RH: At baseline, an average of 2.62±0.33 was found while the three-month average was 0.26±1.21. The difference was statistically significant, (p<0.001).

RW: Average values were 3.56±0.74 and 1.10±1.30 at baseline and three months respectively. A significant difference was found, (p<0.001).

GT: Median values were 0.55±0.24 and 1.32±0.60 respectively at baseline and three months, with a significant difference between time-points, (p=0.003).

KTW: The average KTW was 1.69±0.86 at baseline versus 1.60±0.65 at three months. The difference was not significant, (p=0.546).

CAL: At baseline 4.48±0.74 was detected versus 3.23±1.17 at three months. The difference was statistically significant, (p=0.02).

PD: The average values at baseline and three months were 1.62±0.58 and 1.70±0.41 respectively. The difference was not significant, (p=0.930).

Please refer to table 6.

Discussion

Periodontal Biotype:

The present study compares the root coverage, resolution of sensitivity, KTW gain, PD, CAL, RW and changes in GT using an acellular dermal matrix allograft in two different biotype populations, namely a thin versus thick gingival biotype at three months.

The primary outcome of the present study was root coverage, expressed as the average of the root coverage achieved for each of the two test teeth included in the study. The root coverage was expressed both as an arithmetic mean and as a "capped" average, meaning that the highest acceptable value in this case was 100%. The available literature on this topic suggests that there is a tendency for thinner tissues to perform less well in a variety of periodontal surgical procedures, including mucogingival surgery. Nevertheless, in the present study no statistically significant differences between the two groups were found for the mean root coverage or for the "capped" root coverage. The study by Baldi et al.,[27] for instance, reports that there was a positive correlation between the thickness of the flap and the amount of root coverage that was achieved with the coronally advanced flap. The outcome of the root coverage procedure, as in the present research study, was assessed at three months post-surgery and the authors found that the probability of complete root coverage increased when the baseline thickness of the flap was greater than 0.8mm. In fact, the achieved root coverage was always 100% when the flap was thicker than 0.8mm, while when the flap thickness was equal to 0.8 mm, 2/3 defects achieved complete root coverage. On the other hand, an initial flap thickness below the baseline cut-off value of 0.8 was often associated with residual recession after surgery. Despite the fact that there is no statistically significant difference between groups in the present study, clinical impression is that there is a trend towards higher value of root coverage in the thick group versus the thin, in accordance with the results reported by Baldi et al. The possible difference might also be due to other factors, for instance the methodology of measuring the flap thickness. In the present study, we used a digital caliper and an endodontic reamer with rubber stop. In the Baldi study, however, the thickness was measured with a specific modified Ivansson gauge at the time of surgery and rounded to the nearest 0.5 mm. This method might be less precise and allow for greater subjectivity. In terms of instrumentation, the methods section of the study is quite vague. It does not appear, however, that the authors made use of any micro-instrumentation, such as tunneling knives or microblades, or magnification systems consistently with the time in which the report was published. The authors used silk sutures, which today are largely replaced by monofilament, absorbable sutures for this type of procedure, (such as the one used in the present study, poliglecaprone). The use of microsurgical techniques has been advocated in periodontal plastic surgery as it was found to yield higher root coverage in comparison to traditional macrosurgical approaches. [79] Furthermore, the Baldi study used a coronally advanced flap alone to achieve root coverage, while in the present study an allograft was used, which might provide thickness and therefore enhance the stability of the gingival tissues. Clearly, the question arises as to whether the superior result in the thin tissue category in our study might possibly be explained by the use of a graft material, and not necessarily an allograft. This is all the more interesting because all the studies that have been conducted to investigate the association between flap thickness and root coverage are associated with coronally advanced flaps alone and do not include placement of grafts. [52], [55]

Recently, an article [80] has been published which investigates the effect of biotype on root coverage with an autogenous connective tissue graft. Similarly, to the results of our study, there were no differences in root coverage between the gingival biotype categories. However, there are some very significant methodological differences between the Kahn study and ours. First of all, the concept of “biotype” was not extended, as is the case in the present study, to include the quantitative measure of flap thickness. The biotype was assessed visually and classified as either a thin or thick biotype, solely on the appearance of the tissues. Therefore, the categorization of “thick” or “thin” biotype was not related to a measure of gingival thickness and a range of different thicknesses could be included in each category. Interestingly, the mean thickness of the gingiva in the thin group was higher than that of the thick group at the base of the mesial papilla. Furthermore, the measure of gingival thickness was performed at the base of the papillae, (mesial and distal), and not apical to the gingival margin area, as is the case in the present study. This specific location at the base of the papillae is less representative of the actual thickness of the tissues intraoperatively, and therefore may have less of an impact on the healing and outcome of the surgery. Furthermore, after the surgery the values of gingival thickness increased very little, despite the use of an autogenous tissue graft. Therefore, this specific measure may also be a poor predictor of the future stability of the gingival margin because it would not indicate if the increased thickness after the surgery contributes to stabilize the position of the gingival margin. Finally, the measurements were recorded without the use of a stent. Despite these differences, the results of the present study are reasonably similar to the ones presented above: 70% complete root coverage in the thin group, (versus 63.6% in the present study), and 77.8% in the thick group, (versus 80% in the present study). The difference in baseline gingival thickness and in the location of measurement could possibly account for these slight differences, (1.78±0.23 mm was the average gingival thickness in the thin group reported by Kahn et al., while in the present study it was 0.59±0.14; 1.84±0.26 was the average thickness for the thick group in Kahn's paper, while it was 0.93±0.08 in the present study). Another interesting study from Belgium [81] compared the stability of a subepithelial connective tissue graft used for buccal contour augmentation after implant placement in thin versus thick biotype. The stability in a horizontal direction was assessed using an ultrasonic device up to 9 months post connective tissue graft placement. In this case, the biotype was assessed with probe transparency. Again, there was no numerical definition of biotype as was the case in the previous study. On average between the two groups only 0.1 mm of horizontal tissue shrinkage occurred after 9 months, however interestingly the thick biotype group had a higher degree of soft tissue volume contraction. Similarly, the thick biotype had a gain in soft tissue volume of 86.1% versus 98.9% in the thin biotype group, although the difference was not statistically significant. This study suggests that in the medium-term the stability of the tissue thickness would not differ between a thicker or a thinner biotype. However, we must bear in mind some important differences with the present investigation: firstly, in this case the investigation involves an autogenous graft on the buccal aspect of an implant site; secondly, the mucosal thickness at the implant sites was significantly higher in both thin, (1.02±0.21 mm), and thick, (1.32±0.31 mm) groups, with a GT difference between biotypes of only 0.3 mm. The relatively small difference in GT between the two groups could possibly explain the absence of observed difference in outcome. Nonetheless, the study seems to confirm the observations of Kahn et al. [80] and indicates no apparent difference in outcome between thick or thin biotypes.

Root Coverage:

A 2010 study by Barker et al. [82] compared two different acellular dermal matrix products for root coverage in a split-mouth study design. The study had a 6-month follow-up and employed similar surgical techniques as the present study. Barker and colleagues found a mean root coverage at 6-months of 83.4% with ADM. Although the 3-month root coverage is not directly reported, it can be calculated with the published data and would equal 72.3%. This value increased to 87.3% when only class I defects were considered, but the average root coverage was somewhat decreased by the inclusion of Miller class III recession defects. The vertical recession height did not change significantly between 3- and 6 months. The value reported by Barker is slightly lower than the root coverage in the present study, (100.00±28.00 for thick biotype and 92.36±62.8 for thin biotype, leading to an average of 96.18%), and this could be explained by the inclusion of Miller III recessions in the former study and the potential change between 3 and 6 months, which has not yet been evaluated in our study.

Shepherd and co-authors [83] published another article in 2009, which investigated the effect of PRP on the outcome of root coverage when using ADM combined with coronally positioned tunnel, (CPT). Defects were Miller class I and II single recessions, followed up to 4 months. The mean root coverage achieved for the ADM+CPT group was 70±2.6% at 4 months, again lower than the root coverage achieved in the present study. On the other hand, the defect coverage in the test group, (ADM+PRP), achieved 90±16% root coverage. The authors indicate that worst results were achieved in the mandibular arch, possibly due to excessive flap tension which could not be overcome with the CPT. In contrast with our study, Shepherd et al. used a CPT instead of a flap approach. Previous studies [70] have found better results when ADM is combined with a CAF rather than a CPT, possibly because the tunnel might be more technically challenging or because of the inadequate flap release, insufficient to guarantee a stable gingival margin location when the tunnel is used.

The present study indicates that there was no increase in KTW in either group at three months. This finding is similar to the results of other studies [82], [84], which found no impact of ADM on KTW. On the other hand, Harris et al., [72] found that in the long-term, (4 years), defects treated with ADM gained an average of 0.7 mm of KT while in the short-term, (4 months), the average gain was 1.0 mm. A possible explanation for this difference might lie in the methodology, because in the aforementioned paper the measurements were made using a Williams probe and rounding to the nearest 0.5mm while in the present study they were recorded using a rubber stop on the probe and measuring with a digital caliper.

The comparison between groups yielded a difference in KTW at both baseline and 3 months. This is in agreement with the observation that thicker tissues tend to be associated with a larger band of keratinized tissue. [44]

Gingival Thickness:

Gingival thickness and its variations are a critical parameter to consider in the present study. In 1989, Allen and Miller reported that the CAF achieved excellent results when the initial flap thickness was "adequate", although no numerical value was proposed [26]. A threshold value was only later identified by Baldi et al. in 1999, and this contributed to shift attention on gingival thickness as an important predictor of successful root coverage. In the present study, the flap thickness is one of the most important outcomes and is compared between and within the groups. The baseline thickness was 0.94±0.08mm and 0.59±0.14mm in the thick and thin groups respectively. The increase at three months was very similar in both groups and amounted to approximately 0.6 mm, suggesting that the early healing process did not differ markedly between thick and thin biotypes. This finding is very interesting and should be followed up and compared between groups in the future to determine whether there will be any long-term changes.

The gain in thickness in the present study was similar to the value reported by Ahmedbeyli [67] et al., (about 0.7 mm), although this study had a 12-month follow-up. Shepherd et al. [83] found a change of about 0.4 mm between baseline and 4 months in both the test and control groups when measurements were made using an ultrasonic meter at the base of the sulcus. In the study by Woodyard et al., [85] the authors compared the increase in GT obtained with ADM versus a simple coronally advanced flap. The results indicated an increase of 0.4±2.6 mm in ADM group, (versus only 0.03 mm gain in the CAF group), measured with an ultrasonic meter. At 6 months, the ADM group displayed a root coverage of 99% versus only 67% achieved using the CAF. The data in the present study is therefore consistent with the available literature. Woodyard et al. [85] attributed the higher degree of root coverage achieved in the ADM group versus the CAF to the greater increase in gingival thickness provided by the material. Although in our study we found no statistically significant difference between the two groups, there is undoubtedly a tendency in the thick group towards a greater root coverage. The long-term effect of gingival thickness on the stability of the position of the gingival margin has yet to be determined.

Clinical Attachment Level:

Regarding CAL, there was not a statistically significant difference between groups at 3 months, (3.38±1.21 mm for the thin group, while in the thick group CAL was on average 2.22±1.41 mm; *p* value 0.057). This value is slightly greater than the one reported by Woodyard, [85] (1.21±0.4 mm), but similar to the values reported by Henderson [84], (2.05±0.6 mm in test group and 1.95±0.5 mm in the control group), Aichelmann-Reidy [86], (2.1±1.0 mm) and Harris [72], (2.61±0.74 mm). CAL is measured as the distance between the CEJ and the bottom of the sulcus/ pocket or in alternative the sum of the PD and RH. The greater increase in CAL in the thick group probably results from a combination of PD reduction and RH reduction. Both PD and RH were not significantly different from the thin group, however when combined they could determine a significant difference.

Dentinal Hypersensitivity:

In the present study, patient-reported outcomes were reported and more specifically sensitivity was evaluated before and after surgery. The study was not designed to evaluate the effect of root coverage using ADM on dentinal hypersensitivity but rather to compare the two groups at baseline and the changes that they experienced throughout the course of the study. There was no difference in baseline sensitivity, suggesting that other factors may be responsible in determining the presence and intensity of hypersensitivity. By the same token, there were no differences in the amount of change in hypersensitivity between groups at the different time-points. The available data suggests that flap thickness and hypersensitivity are unrelated.

Complication Rate:

Similarly, the two groups were compared for incidence of complications at 1 week and 3 weeks. Interestingly, there was no difference between groups at either time-point despite the hypothesis that the thin group might have a higher complication rate. Thicker gingival tissues have two main characteristics which provide an advantage during the healing process; firstly, the thicker ECM and collagen fiber component make the tissues more resistant to trauma and prevent collapse. Furthermore, a well-represented vascular compartment is essential during healing because it provides adequate nutrient supply with oxygenation and removal of toxic by-products. Furthermore, it carries the proteins and growth factors that orchestrate the healing process. The blood supply to the gingiva comes mainly from the periosteum and the underlying bone and PDL vessels, which anastomose to form a communicating reticulum. When a split-thickness flap is elevated, the vessels above the periosteum are severed and the flap relies heavily on collateral vascularization for support. If the blood supply to the flap is insufficient, necrosis may occur. [55] A thin flap could be compared to a split-thickness flap and will similarly depend on collateral circulation for its survival. Therefore, it would be more intuitive to expect a higher rate of flap necrosis, epithelial ulceration [87] and contraction in comparison with thicker tissues. It is possible, however that even in the thin group the tissues were thick enough to avoid this complication; a threshold value of thickness below which tissues are more susceptible to necrosis has yet to be determined. Furthermore, all procedures in the present study were undertaken with a microsurgical approach, which could explain reduced trauma to the tissues.

Limitations and Future Perspectives:

The main limitation of the present study is its short follow-up. Although it provides valuable information regarding the short-term healing pattern in thick and thin biotype groups, it would nonetheless be clinically relevant to determine whether a difference between the groups for each parameter arises with a longer follow-up period. In terms of gingival thickness and volume stability, it would also be relevant to ascertain whether there is a difference in the shrinkage rate between the two groups and whether this translates into a change in root coverage over time. The importance of thicker tissues on the rate of recession recurrence remains yet to be confirmed, although it is well known that recession is more commonly observed in thin gingival biotypes and therefore that thin biotypes are susceptible to gingival recession. [44]

This study responds to many of the recommendations of the AAP 2015 consensus report [88] on periodontal soft tissue root coverage procedures, for instance expanding periodontal plastic surgery research protocols to include multiple recession defects and determining the importance of local factors such as periodontal phenotype. Furthermore, it includes patient-reported outcomes. Nonetheless our results group Miller class I and II recession defects, while the consensus statement suggests that conducting research which separate these two categories might help shed light on potential differences between the two disease entities. These could have thus far been masked by the fact that most research protocols unite the two disease categories. It would be relevant in the future to stratify results by Miller class, so that the efficacy of ADM could be compared between Miller class I and II defects for instance.

At present, although gingival thickness is widely recognized as a factor closely related to root coverage, it has not been possible to identify a specific threshold. Hwang and co-authors [55] write that "it is not possible to conclude anything definitive regarding minimal tissue thickness, or critical thickness threshold for predictable root coverage outcome" and they attribute this difficulty largely to the significant heterogeneity and disparities among the (few) available studies on this topic. In the future, efforts should be made to determine the effect of tissue thickness on stability of root coverage over time and the long-term follow-up of the present cohort of patients could help to shed light on this particular aspect. Furthermore, the long-term results of the present study could help define a threshold specific to ADM associated with successful outcomes in periodontal plastic surgery: this could be a very useful tool in the decision-making process for the clinician.

Conclusion

The present study supports the use of ADM for the treatment of multiple recession defects in both thin and thick tissue biotypes. Both groups achieved a high degree of root coverage and were associated with a high frequency of complete root coverage after three months. Furthermore, no difference in complication rates was observed and there was no difference between groups in the change of dentinal hypersensitivity. Importantly, the gingival thickness increased by a similar degree in both groups and at three months both groups had a gingival thickness greater than 0.8 mm, the value previously shown [1] to correlate with complete root coverage. The difference in gingival thickness between groups remained significant at three months. This data is not surprising, especially because it is a short-term result measured after three months of healing.

In any event, the data presented in this study suggest that ADM is an adequate material for the treatment of not only thicker tissues but also thin biotypes. This is noteworthy firstly because a lower degree of root coverage has been associated with thinner tissues, and secondly because thin biotypes are more susceptible to recession compared to thicker biotypes.

Long-term results will clarify whether the difference in gingival thickness between groups will have an impact on long-term stability and appearance of recurrent recession. Currently, we know very little of long-term healing of ADM and even less of the differences in healing when thin and thick tissues are compared. Therefore, the long-term observations will provide valuable data for the scientific community.

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APPENDICES

Appendix A: Tables

Table 1: Demographics

		THIN	THICK	
				p
		10/21=47.6%	11/21=52.4%	
GENDER	Female	5/11=45.5%	4/10=40%	0.801
	Male	6/11=54.5%	6/10=60%	
AGE		46.4±15.1	36.4±14.3	0.139

Table 2: Between-group comparison of the clinical parameters between baseline and 3-month parameters. Data are presented as mean, standard deviation, median, inter-quartile range.

Parameters	Thick				Thin				*P* value	Thick				Thin				
	Baseline									3 Months								
	M	SD	MD	IQR	M	SD	MD	IQR	*p*	M	SD	MD	IQR	M	SD	MD	IQR	*p*
GI	0.24	0.22	0.15	0.26	0.16	0.14	0.11	0.19	0.426	0.20	0.14	0.20	0.16	0.18	0.11	0.17	0.16	0.737
PI	0.18	0.17	0.16	0.23	0.28	0.25	0.22	0.31	0.282	0.37	0.28	0.34	0.54	0.39	0.23	0.36	0.31	0.818
RH(mm)	2.63	0.57	2.46	0.75	2.62	0.33	2.53	0.53	0.92	-0.02**	1.47	-0.32**	1.44	0.18	1.18	-0.13**	2.01	0.480
RW(mm)	3.40	0.57	3.30	0.62	0.36	0.74	3.8	1.44	0.557	0.59	1.26	0.00	0.62	1.01	1.28	0.00	2.10	0.557
KTW(mm)	2.69	0.92	2.45	1.24	1.69	0.87	1.42	1.40	**0.018**	2.49	0.96	2.76	2.02	1.58	0.62	1.50	0.94	**0.017**
PD(mm)	2.11	0.72	2.25	1.10	1.62	0.58	1.55	0.96	0.100	1.59	0.50	1.50	0.64	1.64	0.45	1.79	1.00	0.826
CAL(mm)	4.64	0.97	4.55	1.29	4.48	0.74	4.35	0.73	0.666	2.22	1.41	2.03	1.30	3.38	1.21	3.20	2.12	0.057
GT(mm)	0.94	0.08	0.92	0.16	0.59	0.14	0.55	0.24	*	1.58	0.20	1.54	0.18	1.21	0.30	1.25	0.57	**0.004**

*= *p* value is not necessary because group membership was defined by GT at baseline
**= A negative value indicates that the FGM is coronal to the CEJ
M= mean; SD=standard deviation; MD= median; IQR= inter-quartile range

Table 3: Mean Root coverage and capped root coverage expressed as percentages and comparison between groups at three months.

	THICK	THIN	*P*
Mean Root coverage (%)	115.16±54.68	95.12±14.70	0.387
Capped root coverage (%)	100.00±28.00	92.36±62.80	0.282
Data presented as mean ± standard deviation			

Table 4: VAS score at baseline between groups and the change between groups from baseline to 3 weeks, 3 weeks to 3 months and 3 months to baseline.

	BASELINE				Improvement BL-3W				Improvement BL-3M				Improvement 3W-3M			
	Mean	SD	Median	IQR	Mean	SD	Median	IQR	Mean	SD	Median	IQR	Mean	SD	Median	IQR
Thin	3.59	2.44	5	4	1.32	2.69	1	4	3.05	2.56	3	5	1.73	2.33	0	4
Thick	3.36	2.62	3	5	3	2.49	3	4	2.36	1.97	2	3	-0.64	1.81	0	1
p	0.847				0.193				0.562				0.056			

Table 5: Complication rate expressed as a ratio of complications over total number of subjects in each group at 1 week and 3 weeks.

BIOTYPE	1 WEEK	3 WEEKS
Thick	4/10; 40%	2/10; 20%
Thin	3/11; 27.3 %	3/11; 27.3%
P	0.659	1.000

Table 6: Within-group comparison of the clinical parameters between baseline and 3 months.

	THICK									THIN									
	BASELINE				3 MONTHS					BASELINE				3 MONTHS				
	M	SD	MD	IQR	M	SD	MD	IQR		M	SD	MD	IQR	M	SD	MD	IQR	
									p									*p*
GI	.24	.22	.15	.26	.20	.14	.20	.16	0.638	.16	.14	.11	.19	.18	.11	.17	.16	0.722
PI	.18	.17	.16	.23	.37	.28	.34	.54	0.063	.28	.25	.22	.31	.40	.23	.26	.31	0.314
RH(mm)	2.63	.57	2.55	.75	-.24	1.47	-.32	1.44	**<0.001**	2.62	.33	2.52	.53	.26	1.21	-.05	2.03	**<0.001**
RW(mm)	3.42	.57	3.30	.62	.59	1.26	.00	.62	**0.001**	3.56	.74	3.80	1.44	1.10	1.30	.72	2.22	**<0.001**
GT(mm)	.94	.08	.92	.16	1.58	.20	1.53	.18	0.101	.59	.14	.55	.24	1.23	.31	1.32	.60	**0.003**
KTW(mm)	2.70	.92	2.45	1.24	2.49	.96	2.76	2.02	0.485	1.69	.86	1.42	1.40	1.60	.65	1.53	1.10	0.546
CAL(mm)	4.65	.97	4.55	1.29	2.20	1.41	2.03	1.30	**0.002**	4.48	.74	4.35	.73	3.23	1.17	3.14	1.83	**0.02**
PD(mm)	2.11	.72	2.25	1.10	1.59	.50	1.50	.64	0.101	1.62	.58	1.55	.96	1.70	.41	1.90	.63	0.930

M= mean; SD= standard deviation; MD= median; IQR= interquartile range

Appendix B: Figures

Figure 1: photographic representation of an example of the acrylic stent with vertical marks to ensure reproducible probe placement at future visits.

Figure 2: boxplot showing the gingival index between the groups at baseline.

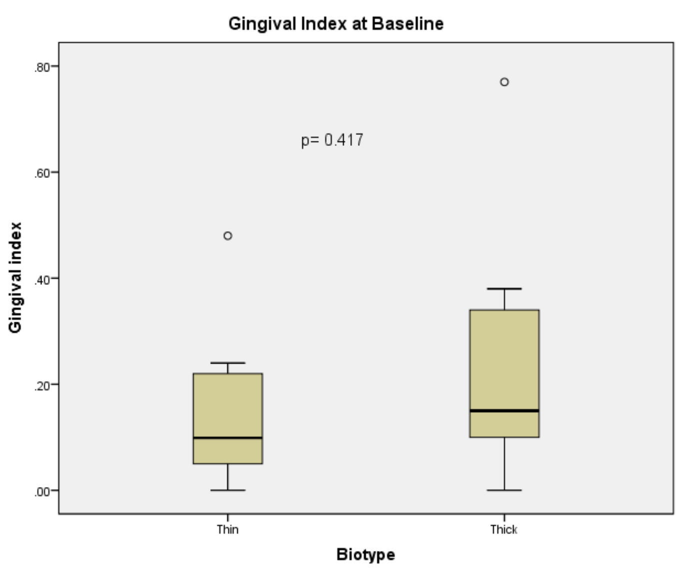


Figure 3: boxplot showing the plaque index between the groups at baseline

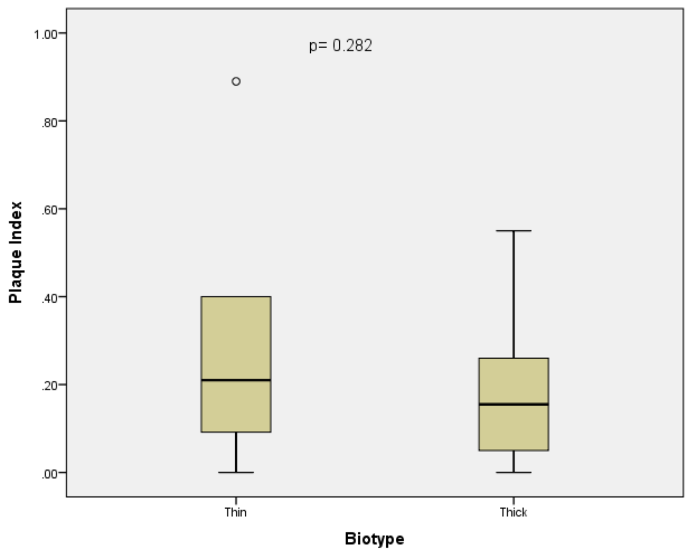


Figure 4: boxplot showing the gingival index between the groups at three months.

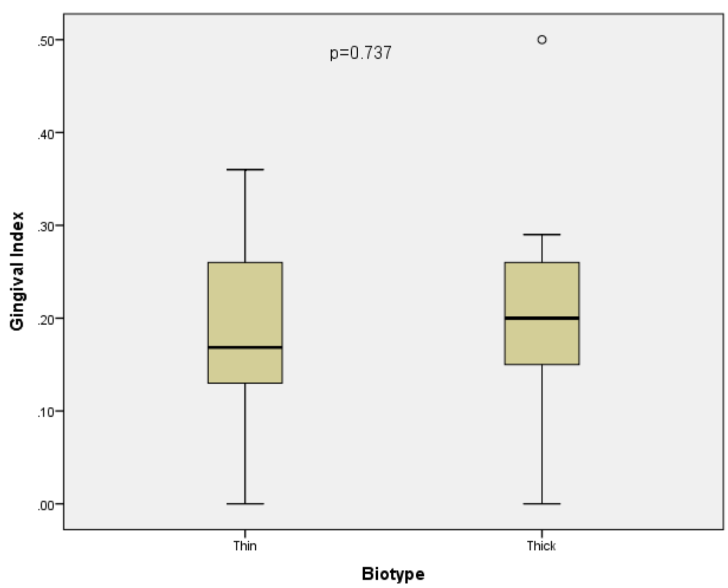


Figure 5: boxplot showing the plaque index between the groups at three months.

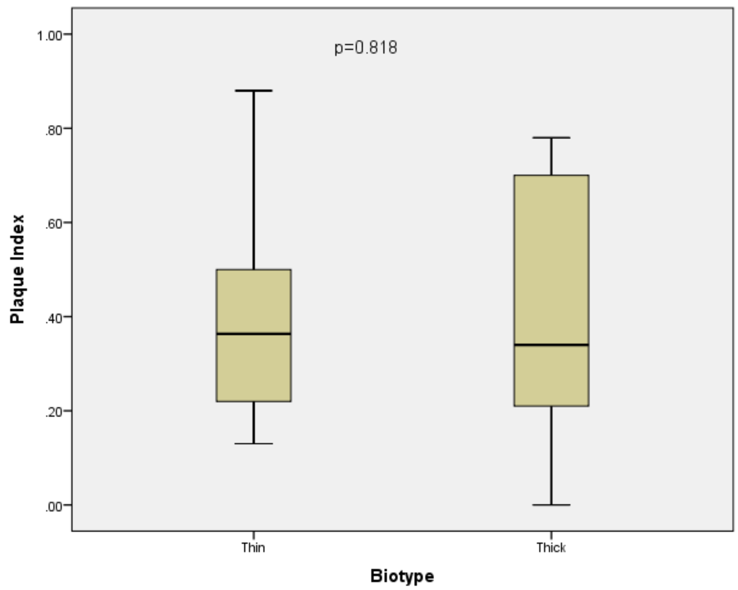


Figure 6: Comparison of GT at baseline between the groups.

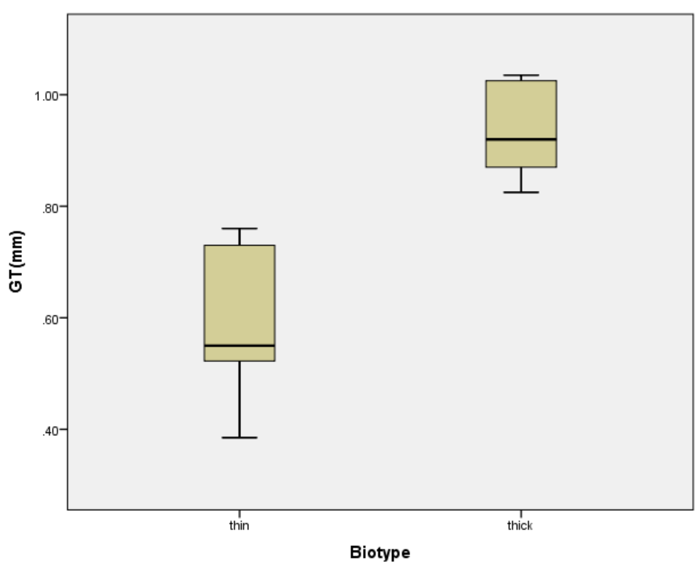


Figure 7: Comparison of GT at 3 months between the groups.

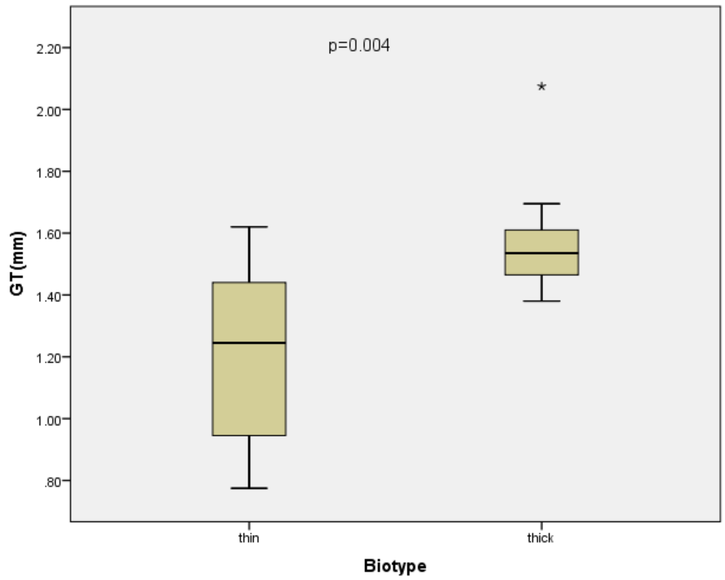


Figure 8: Mean root coverage expressed as percentages at three months.

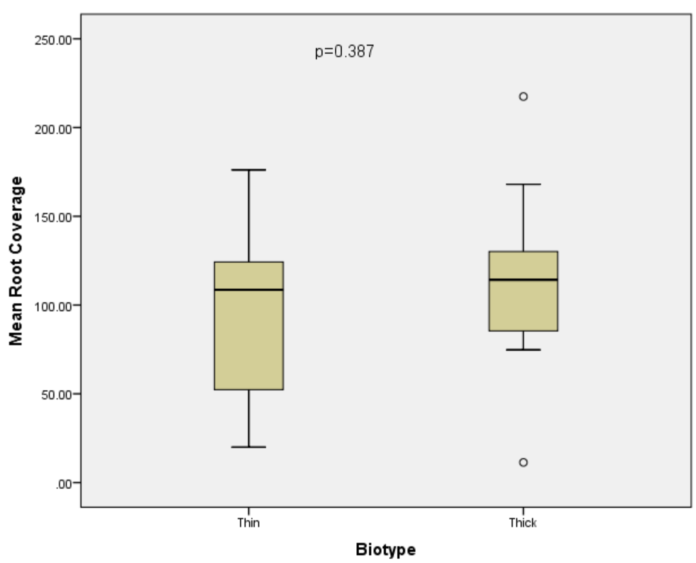


Figure 9: Capped root coverage expressed as percentages at three months

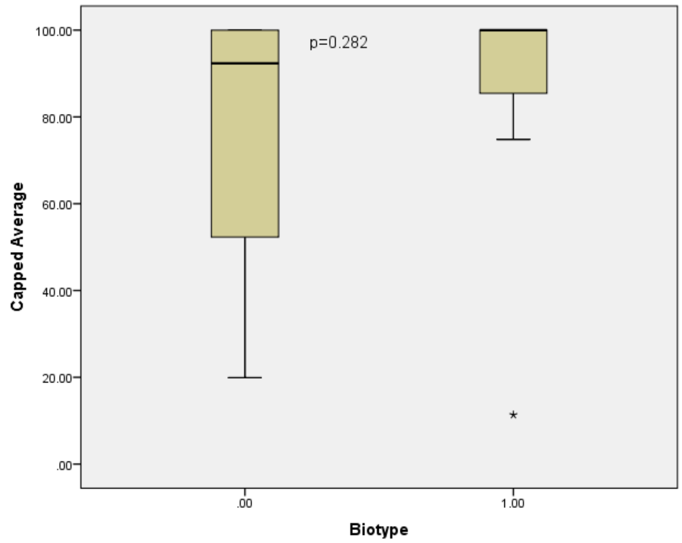


Figure 10: Bar chart showing the percentage of subjects achieving complete root coverage in at least one recession defect at three months in each group.
Thin group: 63.6%; Thick group: 80%

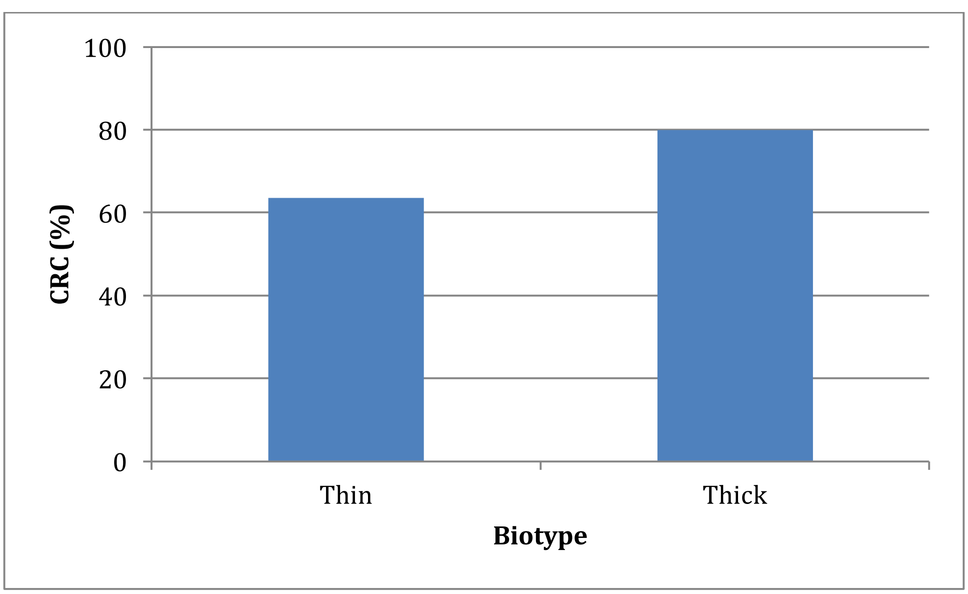


Figure 11: VAS scores at baseline between groups.

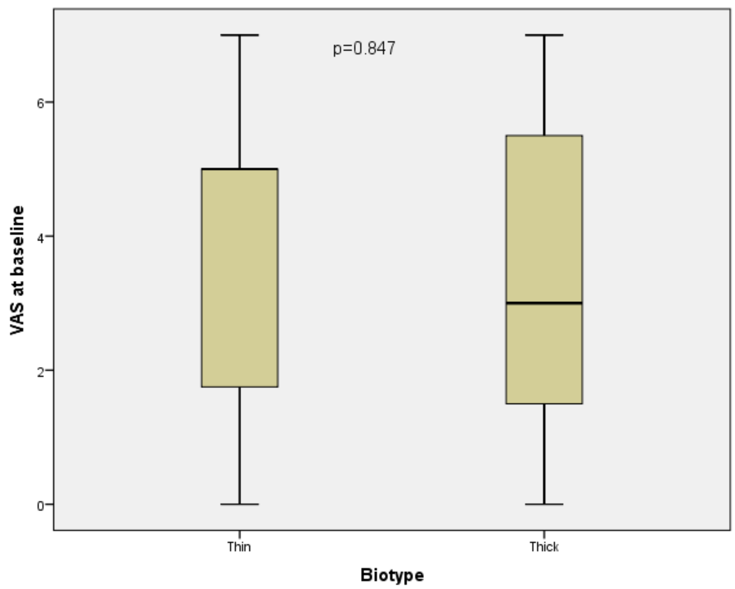


Figure 12: Improvement in VAS score between baseline and 3 weeks.

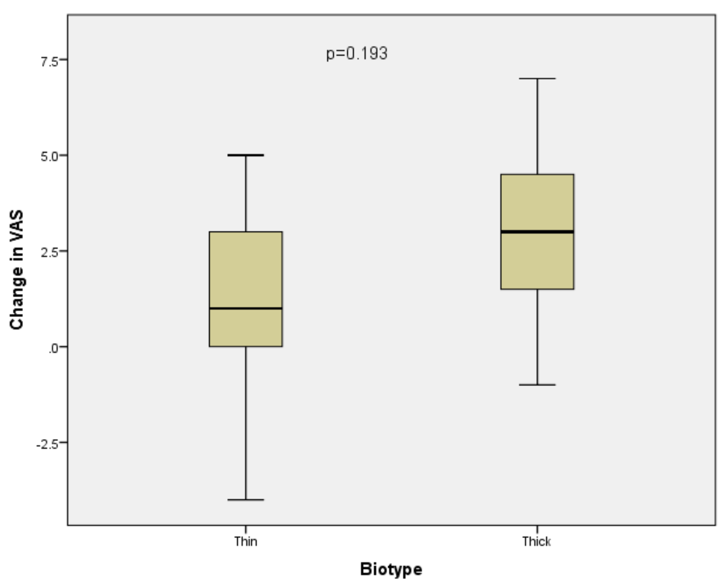


Figure 13: Improvement in VAS score between 3 weeks and 3 months.

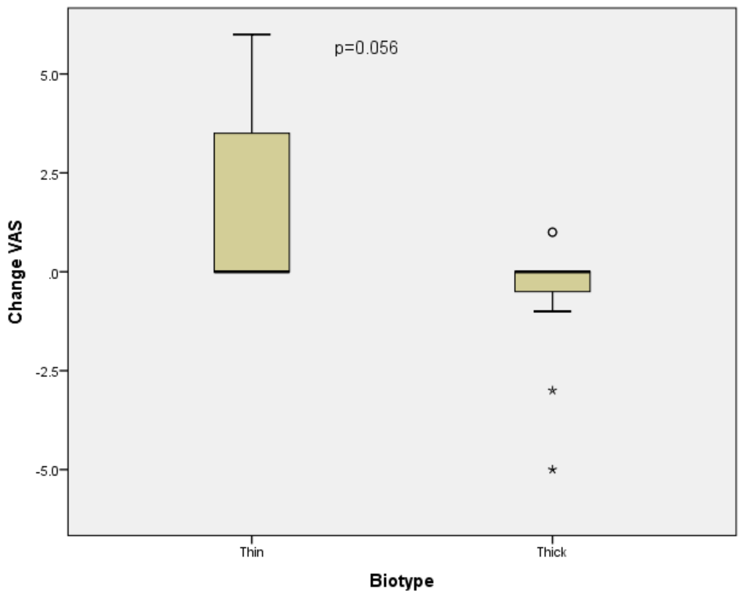


Figure 14: Improvement in VAS score between baseline and 3 months.

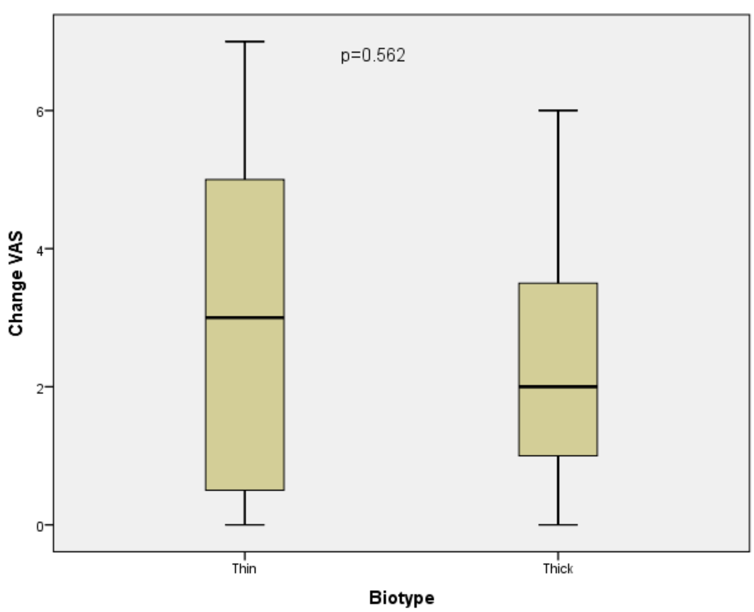
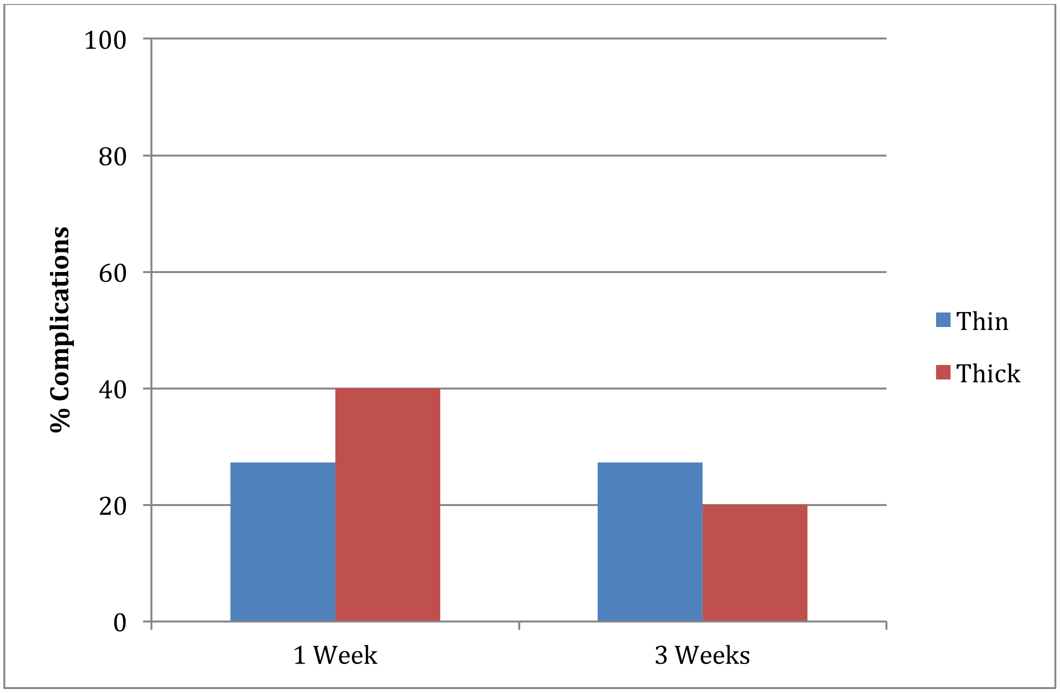


Figure 15: Bar chart representing percent complication-rate at 1 week and 3 weeks between groups. At 1 week, the rate of complications is 27.3% and 40% in the thin and thick group respectively. At 3 weeks, 20% and 27.3% respectively for thick and thin.



Appendix C:

Acellular Dermal Matrix Combined with Coronally Advanced Flap in the Treatment of Multiple Recession Defects in Thin versus Thick Periodontal Biotype Population: A Controlled Observational Study

Patient Satisfaction Survey

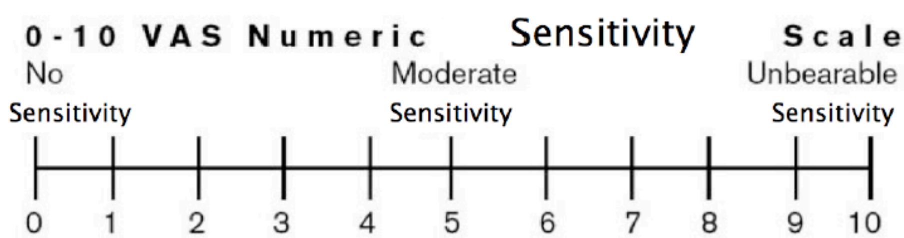
Please answer the following questions with a score of 0 or 1. Key: 1= yes; 0= no

BEFORE SURGERY:

Appearance of the gums -

1. Are you happy with the color of the gum? ☐ Yes; ☐ No
2. Are you happy with the shape and contour of the gum? ☐ Yes; ☐ No
3. Are the teeth sensitive to hot/cold water/air? ☐ Yes; ☐ No

   Is "Yes", please rate it:



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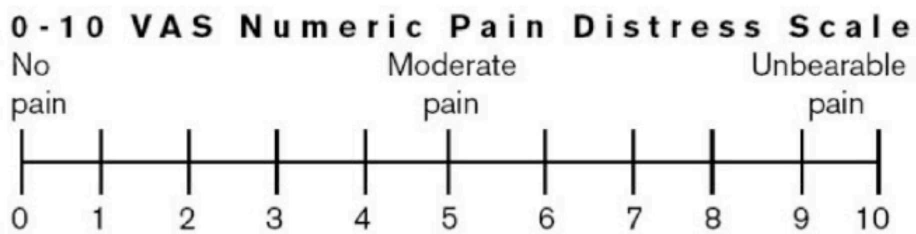
1-WEEK POST-OP VISIT:

During the past week -

1. Did you experience pain in the days following the procedure? ☐ Yes; ☐ No

If "Yes", please rate it.



2. Did you have any complications, (i.e infection, discharge from the surgical area and throbbing pain, bad smell coming from the surgical area and bad taste in the mouth, significant swelling of the face interfering with every day activities)?

☐ Yes; ☐ No

If "Yes", please check the appropriate box:

☐ discharge; ☐ throbbing pain; ☐ bad smell ☐ bad taste

☐ others, please describe ____

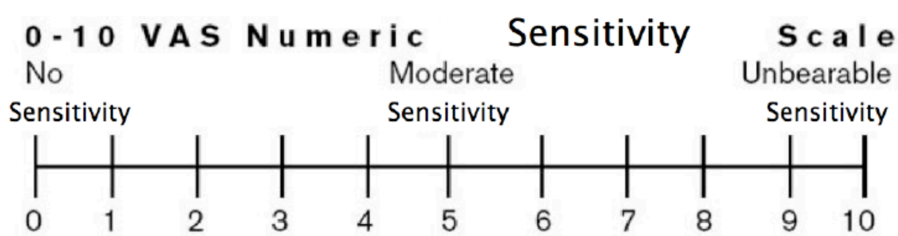
____

Clinical Observations: ____

3-WEEK POST-OP VISIT:

Are the teeth sensitive to hot/cold water/air? ☐ Yes; ☐ No

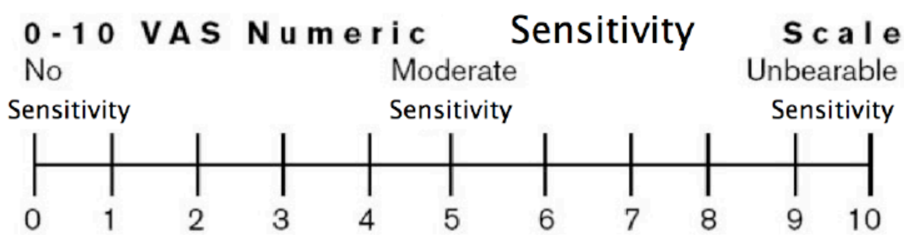
If “Yes”, please rate it:



3-MONTH RE-EVALUATION:

1. Are you happy with the color of the gum? ☐ Yes; ☐ No
2. **Are you happy with the shape and contour of the gum?** ☐ Yes; ☐ No
3. **Are the teeth sensitive to hot/cold water/air?** ☐ Yes; ☐ No

If “Yes”, please rate it:



12-MONTH RE-EVALUATION:

1. Are you happy with the color of the gum? ☐ Yes; ☐ No
2. Are you happy with the shape and contour of the gum? ☐ Yes; ☐ No
3. Are the teeth sensitive to hot/cold water/air? ☐ Yes; ☐ No

If "Yes", please rate it: