GAIN OF KERATINIZED MUCOSA AROUND DENTAL IMPLANTS USING A COMBINATION OF STRIP GINGIVAL GRAFT AND ACELLULAR DERMAL MATRIX – A PROSPECTIVE CASE SERIES.

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ABSTRACT

Several surgical techniques have been described to increase keratinized tissue (KT) around implants and teeth. Despite various methods, the bulk of evidence reported that the use of keratinized autogenous graft (i.e., Free Gingival Grafts (FGG)), harvested from the patient's palate, remained the gold standard in soft tissue augmentation procedures and provided more predictable results. However, soft tissue autograft supply can be limited, and its harvesting is associated by increased patient morbidity. Several clinical studies have demonstrated the effectiveness of using allogenic soft tissue grafts such as Acellular Dermal Matrix Allograft (ADMA) instead of FGG to augment keratinized tissue (KT). This case series evaluates a technique aiming to achieve a change in the tissue quality at areas with insufficient Keratinized Tissue (KT) using an autogenous soft tissue graft (Strip gingival graft (SGG)) with acellular dermal matrix allograft (ADMA).

A total of eight implant sites were treated and assessed for a duration of three months, with two patients (three implant sites) followed up to the six-month evaluation appointment. Key parameters including width of keratinized tissue (WKT), plaque index (PI), gingival index (GI), probing depth (PD), recession (Rec), tissue thickness (TT1 and TT2), esthetics, and patient-centered outcomes were evaluated.

At the three-month postoperative evaluation, no significant changes were observed in PD, GI, and R compared to the screening visit. However, WKT exhibited a significant gain, with an average width of 5.00 mm (SD 0.67 mm, $P<0.05$). Additionally, a significant increase in tissue thickness of 1.06 mm ($P<0.05$) was observed at 2 mm apical

to the gingival margin (TT1). There was a 0.5 mm gain in tissue thickness at 4 mm apical to the gingival margin (TT2), although the difference was not statistically significant ($P>0.05$).

In conclusion, the combination graft of SGG+ADM demonstrated promising results in increasing WKT. Moreover, patients reported minimal morbidity, as evidenced by low post-operative pain measured using the Visual Analogue Scale (VAS).

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LIST OF ABBREVIATIONS

ADM	Acellular Dermal Matrix
ADMA	Acellular Dermal Matrix Allograft
APPTF	Apically Positioned Partial-Thickness Flap
ATS	Activity Tolerance Scale
CTG	Sub-epithelial Connective Tissue Graft
FGG	Free Gingival Graft
FGM	Free Gingival Margin
FPG	Free Periosteal Graft
MM	Mucosal Margin
GI	Gingival Index
GR	Gingival Recession
IRB	Institutional Review Board
KG	Keratinized Gingiva
KT	Keratinized Tissue
KTW	Keratinized Tissue Width
OHI	Oral Hygiene Instructions
PD	Probing Depth
PI	Plaque Index
QD	Once a day
Rec	Recession

SGG	Strip Gingival Graft
STA	Supra-crestal Tissue Attachment
TID	Three times a day
UAB	University of Alabama at Birmingham
VAS	Visual Analog Scale
VP	Vestibuloplasty
WKT	Width of Keratinized Tissue
XCM	Xenogenic Collagen Matrix

INTRODUCTION

To this date, the topic of whether or not a minimum amount keratinized mucosa (KM) is needed around dental implants to preserve health remains controversial. Some authors proved that KM is not an important factor for implant maintenance (Frisch et al., 2015; Wennstrom & Derks, 2012; Kennedy et al., 1985). However, several studies showed that sufficient KM around implants is necessary for stability of peri implant tissue health and implant survival rate (Artzi et al., 1993; Ladwein et al., 2015; Bouri et al., 2008). It was proven that adequate KM around implants allowed for better plaque control by decreasing discomfort from abrasive oral hygiene practices and create a healthy environment for restorative treatment (Block & Kent, 1990). Furthermore, increase in patient satisfaction as well as decrease in complication rate, risk for buccal recession, plaque and bleeding levels are all stated benefits of adequate tissue around implants (Weisner et al., 2010).

Several surgical techniques have been described in order to widen the zone of KM around implants, including apically positioned flap/vestibuloplasty, free gingival graft (FGG), subepithelial connective tissue graft (CTG), acellular dermal matrix allograft (ADMA), xenogenic bilayer collagen matrix, and newer cell-engineered grafts (Scheyer et al., 2015). These techniques have been tried in periodontal plastic surgeries with different success rates.

Despite various methods, the bulk of evidence reported that the use of autogenous grafts (i.e. FGG and CTG), harvested from the palate (fully keratinized tissue), remained the gold standard in soft tissue augmentation procedures and provided more predictable results (Scheyer et al., 2015). Karring et al., 1975, described the process by which autogenous grafts are able to increase the width of keratinized tissue. Histologically, he demonstrated that the characteristics of gingival tissue (and epithelium) are genetically determined by induction from the underlying CT and not a result of

functional adaptation. Therefore, a palatal tissue graft, both in the form of CTG or FGG, carries the genetic information to the recipient site and induces epithelial differentiation and keratinization. Although FGG results in less tissue contraction and shrinkage, the esthetic outcomes are usually less favorable. To accommodate for graft shrinkage a tendency to harvest a larger graft which most of the times leave a large wound area to heal by secondary intention. This is usually associated with increased postoperative patient morbidity, discomfort, and increased risk of hemorrhage from donor site.

To decrease extensive autograft harvesting, Han et al., 1993, described the "The Strip Gingival Autograft Technique" (SGT). This technique utilizes harvesting thin strips of FGGs and then placing them parallel to each other, where one strip is placed in a coronal position and the other one is placed in a more apical position leaving exposed periosteum between both strips. This showed an increase in the width of keratinized tissue with significant decrease in patient morbidity. Disadvantages of this technique include high technical demands and longer healing time due to exposed periosteum between the strips that is healing by secondary intention (Urban et al., 2015).

Acellular dermal matrix (ADM) allograft, originally used for treating burn wounds (Wainwright et al., 1996), has been introduced as an alternative for autogenous soft tissue grafts to treat gingival recession (Allen, 2006) and to increase the width of keratinized tissue around teeth and implants (Wei et al., 2000). ADM is a freeze-dried matrix that is free of epithelium and cellular components where types I and III collagen bundles and elastic fibers are its main components (Cummings et al., 2005; Scarano et al., 2009). This allograft acts as a bioactive scaffold that allows the migration of fibroblasts, epithelial and endothelial cells and can integrate into host tissue via vascular channels of the recipient sits that allow revascularization of the ADM (Jhaveri et al., 2010). Several clinical studies have shown the effectiveness of using ADM instead of FGG to augment peri-implant KM (Gapski et al., 2005). Main advantages of ADM compared to FGG are single surgical site, no donor site intervention leading to less patient morbidity and discomfort and good tissue blending with superior esthetic outcomes (Wei et al., 2000; Agarwal et al., 2015;

De Resende et al., 2019). However, a high shrinkage percentage was reported when using ADM as a sole material compared to other grafting options.

The aim of this study was to evaluate the outcomes of adding a strip gingival graft technique with the acellular dermal matrix to treat mucogingival defects around dental implants.

LITERATURE REVIEW

Summary of Wound Healing

The initiation of the haemostatic phase occurs in response to tissue injury, including defects resulting from periodontal surgery (Dickinson et al., 2013). The formation of a blood clot, derived from blood coagulation, rapidly seals the defect site. Platelets, along with other blood-derived cells such as neutrophils and red blood cells, become activated and aggregate within the blood clot, also known as the blood coagulum. A newly formed fibrin meshwork, comprising the extracellular matrix, forms the major component of this clot, which also includes proteins like fibronectin and vitronectin for cell adhesion (Clark et al., 2004; Reheman et al., 2005). This conglomeration of cells and the fibrin-rich matrix is commonly referred to as the "provisional extracellular matrix" since it will later be replaced by granulation tissue. The formation of the blood clot also triggers the recruitment of inflammatory cells to the defect site.

This inflammatory phase runs parallel to the haemostatic phase, with neutrophils being attracted by chemokines, the complement system, and peptides released during fibrinogen cleavage (Kolaczkowska & Kubes, 2013). Endothelial cells regulate the extravasation and migration of cells into the surrounding tissue (Shi & Pamer, 2011; Kolaczkowska & Kubes, 2013). Neutrophils typically appear at the defect sites within one hour, while monocytes arrive within 24 hours. Neutrophils contribute to wound cleaning by eliminating invading bacteria and releasing proteases before being phagocytosed. Macrophages, on the other hand, are a diverse population that can exhibit both inflammatory and anti-inflammatory phenotypes (Mantovani et al., 2013; Novak & Koh, 2013). In general, the resolution of inflammation is a controlled process involving lipid mediators (Serhan et al., 2008). The transient but crucial inflammatory process sets the stage for subsequent steps in the anabolic phase of new tissue formation.

This phase begins with the development of "granulation tissue," characterized by a highly vascularized structure composed of fibroblasts and an extracellular matrix. The transition from the catabolic to the anabolic phase involves the activation of three key cell types: endothelial cells, fibroblasts, and epithelial cells. The cellular origins of these components are partially understood. Endothelial cells contribute to the formation of new capillaries and can arise from existing blood vessel endothelial cells or circulating endothelial progenitors (Potente et al., 2011). Fibroblasts can originate from connective tissue in the wound edges, monocyte-derived fibrocytes, vessel-derived pericytes, and possibly through epithelial-mesenchymal transition (Grieb et al., 2011; Reilkoff et al., 2011; Weber et al., 2012). Epithelial cells primarily come from keratinocytes at the wound edges, but in certain cases, stem cells from hair follicles can contribute to re-epithelialization (Blanpain & Fuchs, 2009; Cordeiro & Jacinto, 2013). A subset of fibroblasts adopts a myofibroblast phenotype resembling smooth muscle cells, which facilitates wound closure and is vital for the healing process (Tomasek et al., 2013; Klingberg et al., 2013).

The long-term remodeling phase, leading to scar tissue formation, begins with the resolution phase. Apoptosis occurs in most myofibroblasts, fibroblasts, endothelial cells, and macrophages, resulting in a collagen-rich extracellular matrix with few remaining cells. The specific signals triggering this collective cell death are not yet fully understood (Hinz, 2007). It is important to acknowledge that scar tissue formation not only poses aesthetic concerns but also compromises the biomechanical capacity compared to the pre-injury state. Fibrosis, commonly referred to as scar tissue formation, is a prominent pathological feature in various inflammatory conditions affecting organs such as the liver, lung, heart, kidney, and skin, representing a significant global health burden (Meneghin & Hogaboam, 2007). Consequently, considerable efforts are devoted to the control of scarring, primarily focused on preventing scar formation (Wynn & Ramalingam, 2012). In the context of periodontal wound healing, sub-epithelial connective tissue grafts have demonstrated the potential to yield a dense tissue that contributes to long-term stability in the treated area (Thoma et al., 2011; Santagata et al., 2012). Therefore, it is reasonable

to hypothesize that the presence of a dense and stable soft tissue can provide clinical advantages in periodontal therapy.

Differences between Gingiva around Teeth and Peri-implant Mucosa

The soft tissues surrounding implants and natural teeth have some notable differences. First, around natural teeth, Sharpey's fibers connect cementum to the connective tissue attachment, oriented perpendicularly, while around implants, fibers are oriented parallel and do not insert directly onto the implant surface (Berglundh et al., 1991). Furthermore, the Supracrestal Tissue Attachment (STA), formerly referred to as the biologic width, is the sum of epithelial (0.97) and connective tissue attachment (1.07), is 2.04mm on average around teeth (Gargiulo, 1961). On the other hand, around implants, this attachment is generally longer, ranging from 3-4mm, and one study reported that the average epithelial and connective tissue attachment is 2.14mm and 1.66mm, respectively (Berglundh, 1996). Finally, there is a significant difference in blood supply between teeth and implants, with teeth receiving nutrients from multiple sources, including the periodontal ligament and supraperiosteal blood vessels, while implants rely mostly on supraperiosteal blood vessels.

Free Gingival Grafts

The free gingival graft (FGG) is a soft tissue graft that is obtained from the palate along with the overlying epithelium. It was initially introduced to address the lack or loss of keratinized tissue (Nabers, 1966). The process of healing and the principles that influence the outcome of an FGG have been extensively researched, which has contributed to the high predictability of the procedure (Mormann et al., 1981; Miller, 1987). Several factors have been identified as potential risk factors for the success of an FGG, including improper preparation of the recipient site, inadequate size and thickness of the graft, insufficient adaptation to the recipient bed, and failure to stabilize the graft (Miller, 1987). Studies have demonstrated that during the healing process, the free gingival graft (FGG) experiences shrinkage of about 30% (Yildiz et al., 2019; De Resende

et al., 2018). As a result, a wider graft must be obtained than the area requiring soft tissue augmentation, which can cause discomfort and complications at the donor site following surgery (Griffin et al., 2006; Wessel & Tatakis, 2008). Various researchers have examined the shrinkage of FGG in comparison to other options, such as the apically positioned flap alone or graft substitutes like collagen matrix or acellular dermal matrix (ADM) (De Resende et al., 2018; Lim et al., 2018). These studies have confirmed that all graft materials shrink to a significant extent, with FGG having a greater ability to increase keratinized tissue width (KTW) but with the drawback of a longer surgical time, higher patient morbidity, and a less favorable match in color with the surrounding tissue (De Resende et al., 2018; Lim et al., 2018).

FGG is frequently utilized to restore an appropriate keratinized tissue width (KTW) and gingival thickness when mucogingival defects are present. Agudio et al., in 2016, investigated the long-term effectiveness of FGG by comparing it to untreated sites on the opposite side. They evaluated the stability or coronal migration of the gingival margin, as well as the prevention or exacerbation of gingival recessions (GRs). Their findings indicated that FGG prevented GRs and maintained the stability of the gingival margin, while untreated sites were associated with increased recession depth or the development of GRs.

A randomized controlled trial involving 64 patients compared free gingival graft (FGG) to vestibuloplasty (VP) alone around dental implants with inadequate attached mucosa defined as <1.5mm. The study found that FGG resulted in WKT increases of 2.36mm, compared to 1.15mm for the VP group at 12 months. The authors concluded that FGG is more effective than VP in augmenting WKT around implants (Basegmez, 2012). In another study, 41 implants with inadequate WKT (WKT < 2mm) were treated with FGG or free periosteal graft (FPG) and compared to untreated sites. The study found that WKT increased from 0.98±0.76mm at baseline to 3.63±1.43mm at 6 months after grafting with FGG/FPG. (Baltacıoğlu et al., 2015).

Healing and histological evaluation of FGG

The investigation into the healing and revascularization process of free gingival grafts placed over periosteum in monkeys was conducted by (Oliver et al.; 1968). In this particular study, a recipient bed was meticulously prepared in the anterior region of the maxilla and mandible in monkeys. Buccal attached gingiva in the premolar area served as the source for the free gingival grafts, which were subsequently positioned over the periosteum. Suturing was performed to secure the grafts in place, connecting them to the adjacent interproximal tissue, attached gingiva, and interproximal tissue. The study animals were sacrificed at various intervals, ranging from 0 to 42 days, in order to facilitate systematic observation periods.

Upon histological examination, three distinct phases in the healing process of the free gingival grafts were identified. The initial phase (0-3 days) featured a thin layer of fibrin that separated the periosteum from the graft, accompanied by epithelial degeneration and desquamation of outer layers.

Subsequently, during the revascularization phase (4-11 days), minimal resorption of the alveolar crest was observed, along with fibroblast proliferation between the graft and periosteum. Concurrently, the graft epithelium underwent degeneration and desquamation, while new epithelial cells proliferated from neighboring tissues. By day 11, a dense fibrous union had formed between the graft and periosteum, the granulation tissue had been replaced by fibroblastic proliferation, and the graft had become completely covered by a continuous epithelial layer. Vascularization and capillary ingrowth were noticeable at the base of the graft during this phase.

The subsequent tissue maturation phase (11-42 days) demonstrated further development of connective tissue fibers within the graft, accompanied by a gradual increase in connective tissue density. Keratinization, however, only became apparent at day 28. At the 14-day mark, there was a reduction in the number of vessels throughout the graft's connective tissue, while the connective tissue density continued to increase. Vascular patterns displayed relative stability after day 14, with noticeable changes primarily confined to vessel count and connective tissue density at the 14-day interval.

Reason for increasing the width of Keratinized tissue around implants

There is still controversy surrounding the decision to treat areas lacking keratinized tissue around implants. A review in 2012 included twelve human studies that reported plaque scores for sites with "adequate" (≥2mm) and "inadequate" (<2mm) WKT. Out of these studies, only 5 reported a connection between <2mm WKT and higher plaque scores. Additionally, half of the studies found no significant increase in bleeding scores for implants with <2mm WKT, and 8 out of 10 found no differences in probing depths (Wennström, 2012). However, in a 5-year longitudinal study that evaluated the peri-implant soft tissue health and stability around implants supporting full-arch prostheses, WKT had a clear impact on plaque accumulation, bleeding, and recession. Patients who had WKT <2mm exhibited higher plaque scores, bleeding tendency (BOP), and recession despite undergoing a thorough maintenance program and good oral hygiene (Schrott, 2009; Kim et al., 2009). Few studies have found that implants with <2mm of keratinized mucosa experience more bone loss compared to those with a broader band of KT. Nevertheless, having adequate WKT is important for reducing inflammation and facilitating oral hygiene around implants to prevent long-term bone loss (Bouri, 2008). In clinical practice, it is challenging to maintain consistently good oral hygiene around restorations in the absence of KT (Yeung, 2008).

Acellular Dermal Matrix Allograft (ADMA)

Since 1995, acellular dermal matrix allograft (ADMA), which is human skin tissue donated by individuals, has been used in various applications, such as burns, head and neck reconstructions, urinary applications (bladder slings, pelvic floor reconstruction), orthopedic applications (rotator cuff repair & periosteal replacement), and hernia repair. The allogeneic acellular dermal matrix (ADM) is a processed freeze-dried matrix derived from human dermis, specifically designed to retain its structural integrity, collagen fibers, elastin filaments, hyaluronan, proteoglycans, and basement membrane, while eliminating cellular and epidermal components (Allen 2006). By acting as a bioactive scaffold, ADM

facilitates the migration and adherence of fibroblasts, endothelial cells, and epithelial cells (Jhaveri et al., 2010).

ADMA to increase the Width of Keratinized Tissue

Using acellular dermal matrix allograft to enhance the width of peri-implant keratinized mucosa offers numerous benefits compared to the autogenous-free gingival graft procedure. It eliminates the need for an extra wound in the palatal region and is not limited by the availability of donor tissue. Moreover, reports suggest that the esthetic outcomes of acellular dermal matrix may surpass those achieved with autogenous-free gingival grafts (Wei et al., 2002).

The acellular dermal matrix (ADM) has been applied to various dental procedures, including soft tissue augmentation, keratinized gingiva augmentation, barrier membranes, grafting material for covering amalgam tattoos, and root coverage procedures (Gapski et al., 2005). It is commonly believed that devitalized grafts, when appropriately processed to preserve the extracellular matrix structure, serve as scaffolds that enable the repopulation of fibroblasts, blood vessels, and epithelium from neighboring tissues (Wei et al., 2002; Karring et al., 1975).

Several studies have compared the effectiveness of free gingival grafts (FGG), sub-epithelial connective tissue grafts (CTG), and acellular dermal matrix allograft (ADMA) in increasing the width of keratinized gingiva (KG). One study evaluated 12 patients with 1mm or less of attached gingiva on the facial aspect of mandibular anterior area in a 6-month clinical and histological study and found that while ADMA had better esthetic outcomes than FGG, it was less effective and predictable in increasing the width of KG, with greater shrinkage at 6 months post-surgery (71% vs. 16%). The authors concluded that ADMA had little influence on epithelial differentiation due to the limited amount of gained KG compared to the size of ADMA placed (Wei et al. 2002, Wei et al. 2000). Another study evaluated FGG, CTG, and ADMA in three groups of 15 patients each in a private practice setting for a period of 3 months and found that all three grafts significantly increased the amount of KG (4.1 mm, 3.6 mm, and 4.1 mm, respectively). The

author suggested that the lack of differences in the achieved amount of KG between the various grafts may be due to the periosteal scoring and the partial coverage of ADMA with a flap containing KG in the present study. However, this study had some limitations, including the lack of blinded evaluations, standardized graft sizes and locations, and longer-term follow-up visits (Harris, 2001).

ADM has found expanded applications in dentistry, particularly for soft tissue augmentation to address gingival recession defects and enhance the quality of mucosa surrounding natural teeth and dental implants (Allen, 2006; Wei et al., 2000). In the context of increasing keratinized tissue width, the combination of ADM with autogenous palatal connective tissue graft, utilizing an apically positioned partial-thickness flap (APPTF), demonstrated a 6-month gain of 1.58 mm (Basegmez et al., 2013). Additionally, a separate study reported a 2.20 mm increase in keratinized tissue width at 6 months using ADM with APPTF (Park 2006). ADM offers several advantages over autogenous grafts, including the avoidance of donor site morbidity and an unlimited supply (Fu et al., 2012; Park 2006; Scheyer et al., 2015).

In a study involving a randomized controlled trial, both the free gingival graft (FGG) group and the acellular dermal matrix (ADM) group were found to increase the width of keratinized mucosa around dental implants (Basegmez et al., 2013). However, the FGG group demonstrated superior effectiveness in achieving a mean gain of 2.57 mm compared to the ADM group, which had a mean gain of 1.58 mm after 6 months (Basegmez et al., 2013). Both groups showed a significant reduction in plaque and gingival indices at 6 months compared to baseline (Basegmez et al., 2013). When comparing clinical parameters at 6 months, the FGG group exhibited a significantly lower plaque index than the ADM group, while there were no significant differences in gingival indices between the groups (Basegmez et al., 2013).

In a case report comparing FGG and ADM for increasing the width of keratinized tissue around dental implants, one patient was treated with FGG in the maxilla and ADM in the mandible (Yan et al., 2006). The healing period for ADM was approximately 2 weeks longer than for FGG, and keratinization of ADM was not evident until 6-8 weeks post-

procedure (Yan et al., 2006). At 6 months, the sites treated with ADM had a narrower band of keratinized tissue (2.4 mm) compared to the sites treated with FGG (7.8 mm), but there were no significant differences in clinical parameters such as plaque index, gingival index, probing depths, and gingival recession at 6 months (Yan et al., 2006).

A systematic review focused on patient-reported outcomes of postoperative pain after interventions to increase the width of keratinized mucosa, showing that patients experienced greater pain perception with autogenous grafts compared to alternative grafts (Thoma et al., 2014). Although the reviewed studies compared the use of connective tissue grafts (CTG) to collagen matrix grafts instead of ADM, they emphasized that alternative grafts result in less patient morbidity and better patient-reported outcomes. Additionally, avoiding the need for a second graft harvesting procedure reduced surgical time by approximately 15 minutes (Thoma et al., 2014).

In conclusion, the available evidence suggests that FGG achieves a significantly greater increase in keratinized mucosa width compared to ADM (Bassetti et al., 2017). However, the use of alternative grafts such as ADM offers advantages such as reduced patient morbidity and shorter surgical time (Thoma et al., 2014).

Importance of increasing tissue thickness around implants

Measuring tissue thickness is another crucial aspect when evaluating soft tissue around implants. Some studies suggest that gingival thickness may be a more reliable predictor of gingival inflammation and the risk of gingival recession compared to keratinized mucosa (Müller, 1997; Müller, 2002). In a study involving 482 implant sites with tissue thickness of less than 1mm and 606 sites with thickness exceeding 1mm, surgical open flap debridement was performed. The group with less than 1mm tissue thickness showed a significant apical shift in the position of the gingival margin, whereas no significant change was observed in the group with over 1mm tissue thickness throughout a 16-month period. Thicker flaps are believed to maintain an intact capillary system with improved circulatory potential (Vandana, 2016). In another investigation, crestal bone changes around implants were assessed in relation to initial mucosal

thickness. Group A, with flap thickness below 2mm, exhibited crestal bone loss of 0.6mm±0.5mm, while Group B, with flap thickness above 2mm, showed crestal bone loss of 0.2mm±0.4mm. The study demonstrated a statistically significant higher degree of bone loss when mucosal thickness was below 2mm (Van Eekeren, 2016). These findings suggest that tissue thickness plays a more significant role in predicting implant success compared to other soft tissue dimensions.

Histology of ADMA to increase KT around teeth

In Scarano et al., 2009, histological study, they treated teeth with minimal (<1 mm) keratinized tissue using ADMA. In their study, they took a specimen of the used Alloderm before suturing it to the prepared recipient bed and took a biopsy of the treated site at four minutes after suturing and at 1, 2, 3, 4, 6, and 10 weeks after grafting. Upon examining the ADM prior to suturing, a fibrous reticular connective tissue with collagen fiber bundles displaying hyperchromatic characteristics was observed. No cellular components were visible. The reticular pattern exhibited lacunae filled with a matrix that exhibited metachromatic affinity upon toluidine blue staining. No epithelium was detected. At the 4-minute mark, no significant differences were observed compared to the initial examination, except for the presence of erythrocytes among the collagen fibers.

Generally, numerous macrophages were observed phagocytosing existing collagen fibers during the initial week. Following the first week, fibroblasts, potentially involved in the generation of new collagen fibers, were identified, and some epithelial cells were observed at the periphery of the allograft. By the end of the second week, although inflammatory cells were still present, the numbers of fibroblasts and epithelial cells had significantly increased.

Ultrastructurally, the epithelial cells appeared well-organized, and the cytoplasm of fibroblasts appeared full, likely engaged in the production of non-collagenous and collagenous extracellular matrix. Numerous newly formed small blood vessels were present, particularly in the deepest section of the graft where the ADMG directly

interacted with the host tissues. No significant changes were observed after three weeks. However, a noteworthy decrease in inflammatory infiltrate was observed, and an increase in epithelial cells covering the graft's surface was noted. Newly formed blood vessels were evident on the outer portion of the graft. After four weeks, the graft structure was still recognizable but had undergone drastic modifications. Many collagen fibers had completely resorbed, a basement membrane was apparent, and a substantial increase in the number of blood vessels was observed. Lymphocytes, plasma cells, and histiocytes infiltrated the gingival stroma between residual graft collagen fibers.

Additionally, some newly formed collagen fibers were present. The superficial layers exhibited newly formed stroma resembling granulation tissue, and newly formed vessels were detectable. The preexisting external squamous layer exhibited degenerative necrotic processes and detached from the basal membrane. The deeper layers revealed collagen fibers surrounded by histiocytic cells, occasionally displaying multiple nuclei. Residual collagen fibers could be identified within the cytoplasm of histiocytic cells. At six weeks, a completely re-epithelialized gingiva was evident, and a well-structured basement membrane was observed histologically. Finally, in the 10-week specimens, no inflammatory cells were present, and only a few existing collagen fibers remained. The grafted area exhibited complete re-epithelialization and restoration to its original state.

Strip Gingival Grafts

The strip technique was created with the goal of maximizing the coverage of gingival grafting while minimizing trauma to the donor sites by the rapid epithelization of close wound edges since if donor tissues are removed in strips, they would heal rapidly due to the short distance travelled by the epithelium. Its primary purpose is to increase the width of the attached gingiva rather than addressing gingival recession and covering exposed root surfaces. Essentially, this technique enables the creation of extensive areas of attached and keratinized tissue with minimal patient discomfort.

Clinical observations have indicated that the amount of keratinized attached gingiva obtained through the strip gingival graft technique is approximately proportional to the width of the graft placed at the recipient site. Regardless of the recipient site's width or the positioning of the strip, a condensing effect occurs within three months, leading to the upward movement of the mucogingival junction, aligning with a width similar to that of the donor strip (Han, 1993).

SPECIFIC AIMS OF THE STUDY

The concept of this study was taken from (Urban et al., 2015) prospective case series where they combined the Strip Gingival Graft (SGG) with a Xenogenic Collagen Matrix (XCM) to treat severe mucogingival defects.

The overall goal of this pilot study is to evaluate the changes in tissue quality at areas that lack Keratinized Tissue (KT) using a Strip Gingival Graft (SGG) and Alloderm (ADM) which involves creating a partial thickness mucosal flap with apically positioning of this flap and then using SGG+ADM. The specific aims of study included:

1. Evaluate the amount of Keratinized Tissue increase.
2. Record changes in tissue thickness
3. Asses the color and contour gum esthetics using Pink Esthetic Score (PES)
4. Measure patient-centered outcomes (pain, bleeding, bruising, swelling, and effects on daily activity)

MATERIALS AND METHODS

Study population

Six patients were screened and recruited from the UAB School of Dentistry Graduate Periodontology clinic between August 2022 and May 2023. To achieve the desired sample size of 12 adult patients, another resident will continue the study. The recruited participants will have at least one implant site with a deficiency of keratinized tissue and a loss of vestibular depth. These patients will be selected from the department of periodontology at the University of Alabama at Birmingham. In addition to the lack of keratinized tissue, the patients must meet the specific criteria outlined in (Table 1).

Screening/Baseline

Patients in the School of Dentistry Periodontology Clinic in need of soft tissue grafting treatment were told about the study during the periodontal evaluation appointment and were screened for study entry the same day or an appointment that was scheduled at an alternate time for the screening visit. The demographic characteristics of the included subjects are shown in Table 1. Screening of patients was completed by the participating resident, Dr. Louai Haddad, and the faculty, Dr. Hussein Basma (Committee Chair), and included the review of medical history and confirmation that patients meet inclusion/exclusion criteria (Table 2). A consent form was given to patients to read and discuss.

Study protocols and risks/benefits of study participation were discussed with patients. Surgical procedures involved in the soft tissue grafting and post-operative healing as well as routine and study related visits were discussed as well. A digital intra-oral scan of the surgical treatment site(s) was taken.

Clinical periodontal measurements (Table 3) at the site of interest were performed by the calibrated study examiner, Dr. Hussein Basma, and served as the baseline

measurements to be compared to future measurements. Photographs in a 1:1 ratio of the site of interest will be taken during this visit with an intraoral camera and ring flash.

Table 1. Demographic Characteristics of Included Subjects

Patient #	Age	Sex	Implant Position
1	64	M	B and D
2	60	F	C
3	65	F	B
4	66	M	B
5	68	M	B
6	74	M	B and C

Table 2. Inclusion and Exclusion Criteria

Inclusion Criteria	**Exclusion Criteria**
English speaking	Non-English speaking
At least 18 years old	Less than 18 years old
Must be a patient of the UAB Dental School	Smokers/tobacco users (>10 cigarettes/day)
Able to read and understand informed consent document	Patients with systemic pathologies or conditions contraindicating oral surgical procedures or adversely affecting wound healing

Patients with implants lacking keratinized tissue.	Presence of active periodontal disease
Presence of periodontally healthy, non-carious neighboring teeth, healthy implants or edentulous ridges on either side of the involved site(s)	Previous soft tissue grafting at the site(s) to be treated

Table 3. Clinical measurements and their respective timing of measurements

Clinical measurements	Timing of measurements
Probing depth (PD): A UNC-15 calibrated periodontal probe will be used to measure from the gingival margin to the base of the periodontal pocket at six sites per implant (mesiobuccal, buccal, distobuccal, mesiolingual, lingual, distolingual).	Screening visit, surgical visit, 3 months, 6 months, and 1 year post op.
Bleeding on probing (BOP): A dichotomous variable recording Y/N at six sites per implant after PD measurements	Screening visit, surgical visit, 3 months, 6 months, and 1 year post op.
Plaque Index (PI, Silness & Loe): A modification of the Silness and Loe Plaque Index will be used full mouth. Categories 2 and 3 from the original index will be collapsed into a single category so that examiners only have to distinguish between visible plaque and plaque that cannot be seen but is detectable with the probe. A single score will be recorded for each implant. A single score 0 to 2 will be recorded for buccal and lingual/palatal surface of each implant examined as follows: 0 = No plaque	Screening visit, surgical visit, 3 months, 6 months, and 1 year post op.

1 = A film of plaque adhering to the free gingival margin and adjacent area of the implant which cannot be seen with the naked eye. But only by using disclosing solution or by using probe 2 = Moderate accumulation of deposits within the gingival pocket, on the gingival margin and/or adjacent tooth/implant surface, which can be seen with the naked eye	
Gingival Index *(GI,* Loe and Silness): at surgical site(s). A periodontal probe will be swept around the peri-implant sulcus at a depth of 1-2 mm. The examiner will determine the status of tissue health. A single score will be recorded for buccal and lingual/palatal surface of each implant examined as follows: 0 = healthy tissue 1= mild inflammation but no bleeding 2 = moderate inflammation with bleeding 3 = severe inflammation with a tendency toward spontaneous bleeding	Screening visit, surgical visit, 3 months, 6 months, and 1 year post op.
Width of keratinized tissue (KT): A UNC-15 calibrated periodontal probe will be used to measure KT at implant site(s) at the mid-buccal aspect from the mucosal margin to the mucogingival junction.	Screening visit, surgical visit, 3 months, 6 months, and 1 year post op.
Width of attached tissue (AT): will be calculated by subtracting PD from KT	Screening visit, surgical visit, 3 months, 6 months, and 1 year post op.
Tissue Thickness (TT1 and TT2): at the buccal mucosa of surgical sites will be measured during the surgical visit by horizontal transmucosal probing (sounding the bone) using an endodontic reamer after local infiltration of anesthetic prior to surgical site preparation. TT1 and TT2 will be	Screening visit, surgical visit, 3 months, 6 months, and 1 year post op.

measured at 2 mm and 4 mm from the mucosal margin, respectively.	

Surgical Visit

Confirmation of consent and any remaining questions from patients were discussed.

Study group: Strip Free gingival graft (SGG) in an apical position (the strip graft should be sutured 7mm apical to the peri-implant mucosal margin) + Acellular Dermal Matrix graft (ADM) coronal to SGG around dental implants with lack of (KT)

The examiner confirmed the measurements taken at baseline with relation to width of keratinized tissue (KT) and evaluated thickness of mucosa (TT1 and TT2) as described above. The surgeon then measures the recipient site to determine the size of graft needed and ensures that surgical treatment will avoid any vital structures. The resident who identified and recruited a patient for the study performed the surgery, which was supervised at all steps by the attending faculty, Dr. Hussein Basma. Surgeons followed the standard-of-care procedure for soft tissue as explained below.

Surgical procedure:

Strip Free gingival graft (SGG) + Acellular Dermal Matrix graft (ADM):

A pre-operative photograph was taken prior to the surgical procedure (figure 2). A loading dose of prophylactic antibiotics was dispensed at the time of surgery (Amoxicillin 2g, 30 minutes to one hour prior to surgery). If the patient was allergic to penicillin, Clindamycin 600mg was substituted. Patients were given a 0.2% chlorhexidine solution for 1 minute to rinse with in order to disinfect the surgical site to minimize the potential contamination from extraoral sources. An overview of the surgical procedure is shown in Figure 1.

A local anesthesia with 4% Articaine Chlorhydrate and epinephrine 1:100000, was applied. A horizontal incision is then placed at mid-crest of residual KT. If the regenerative site was in the maxilla and the implants were uncovered the horizontal incision was placed on the palatal side of the KT. Two vertical releasing incisions were followed to allow for apical displacement of the flap. The flap was then elevated with a split-thickness flap beyond the mucogingival junction where it was sutured at this apical position using a mattress (5-0, Monocryl) suture (figure 3). The recipient site should ideally retain intact periosteum that is firmly attached to bone with no loose fibers, no irregularities, and no perforations.

A strip of a free gingival graft was then harvested from the patient's palate. This strip was only 3 mm wide ,1 to 1.5 mm thick and had an appropriate length to cover the full apical extension of the recipient site. The strip was sutured immediately with 5-0 Monocryl sutures (figure 4). 7 mm coronal to the strip (width of the ADM), the periosteal bed was covered with ADM, which was already rehydrated in sterile saline for 10 min, trimmed and customized to fit the available space. Access holes for the healing abutments were created through customized ADM to help increase the retention of that matrix if applicable. The ADM was then stabilized on the periosteal bed with the epithelium side facing upward. The ADM was fixed on the recipient bed using periosteal 5-0 Monocryl sutures (figure 5). Due to the small width of the harvested grafts, the palatal wound margins were approximated with the use of cross chromic gut sutures.

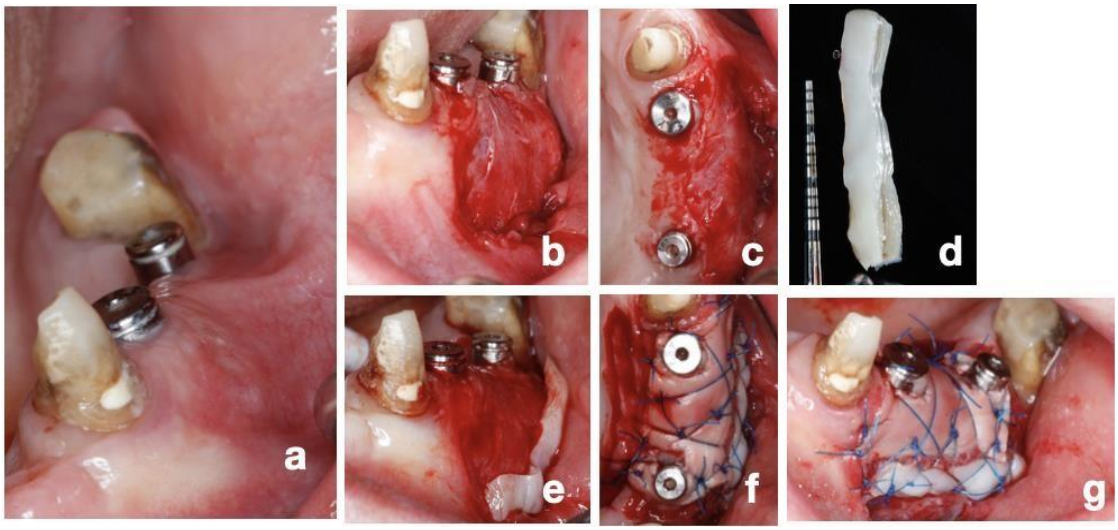

Figure 1: Overview of the surgical procedure.
(a) Pre-operative photo, showing minimal keratinized tissue (Profile view).
(b) Split-thickness flap showing horizontal incisions and divergent vertical incisions (Profile view)
(c) Split-thickness flap showing horizontal incisions and divergent vertical incisions (Occlusal view)
(d) Strip Gingival Graft harvested from the palate with intact epithelium (3 mm width)
(e) Placement of the Strip Gingival Graft on the recipient bed
(f) ADM and Strip Gingival Graft sutured in place (Occlusal view)
(g) ADM and Strip Gingival Graft sutured in place (Frontal view)

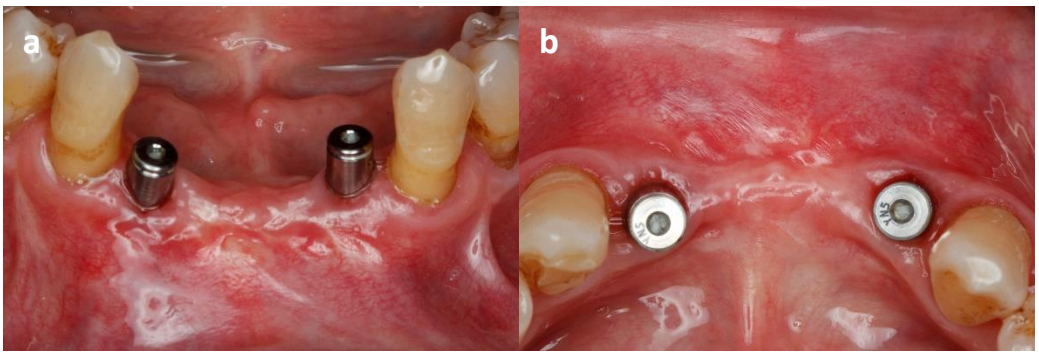

Figure 2. Pre-operative photos. (a) Frontal view. (b) Occlusal view.

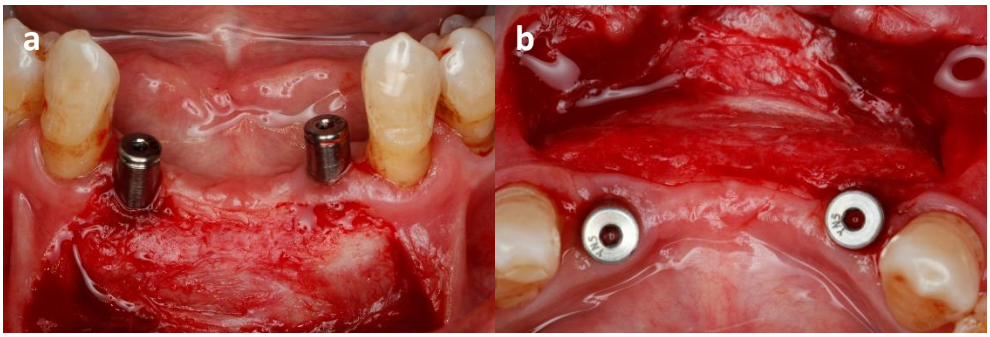

Figure 3. Recipient bed/Flap design. Split-thickness flap showing horizontal incisions and divergent vertical incisions. Frontal view (left) and Occlusal view (right).

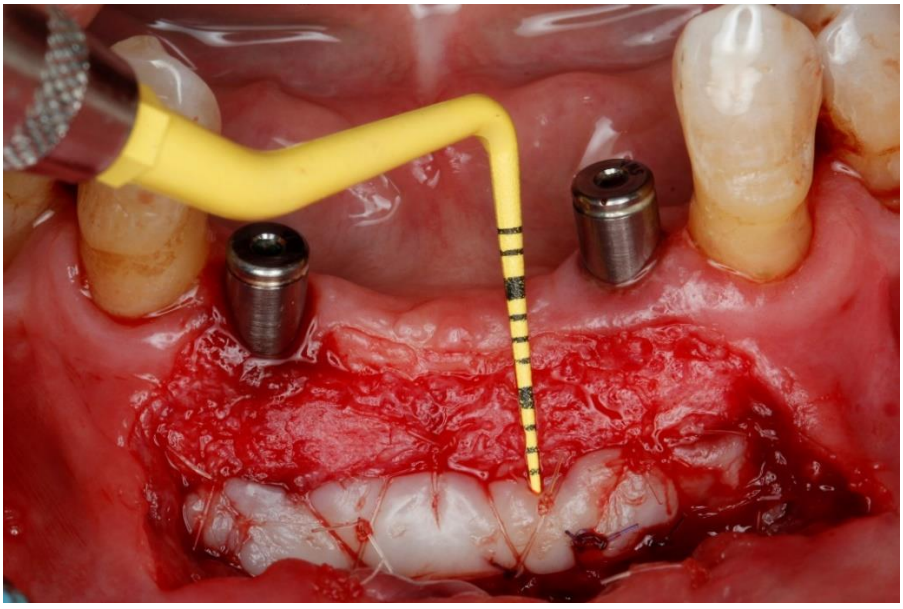

Figure 4. Strip Gingival Graft harvested from the palate sutured 7 mm apical to the implant margin.

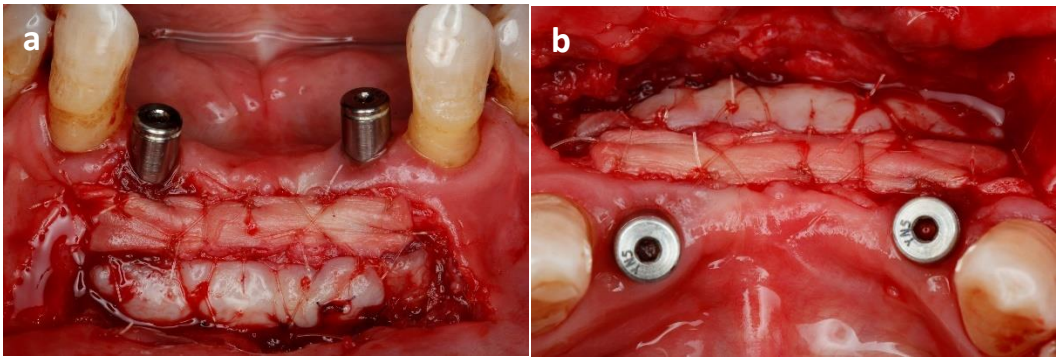

Figure 5. ADM (7 mm wide) and SGG (3 mm wide) sutured in place. (a) Frontal view. (b) Occlusal view.

Post-Operative Care and Instructions

At the end of the surgical visits, detailed instructions were provided to all patients, both verbally and in writing. These instructions covered various aspects, such as the expected discomfort and swelling, dietary restrictions, and at-home care. Patients were advised to be mindful of the surgical sites and avoid them while brushing and flossing. Special emphasis was placed on not pulling the lip to view the surgical area, as excessive muscle tension could potentially hinder the healing process and compromise the success of the graft. For pain management, patients were prescribed Ibuprofen 600mg to be taken as needed, with a maximum daily dosage of 3200mg. Additionally, a five-day course of Amoxicillin 500mg T.I.D was prescribed to prevent infection. To alleviate swelling, patients were provided with an icepack and instructed to apply it for 10 minutes on-and-off within the first 24 hours.

Follow-up Visits

During the course of the study, a subgroup consisting of two out of the initial six patients (with the intention of ultimately expanding the study sample to a total of 12 patients) underwent follow-up visits at multiple time points, including 1 week (figure 6), 2 weeks, 1 month, 3 months, and 6 months. The four remaining patients continued to receive follow-up evaluations until the 3-month appointment. A comprehensive one-year post-operative follow-up will be conducted for all patients, including both the currently

enrolled participants and those who will be recruited for the study. This extended duration will enable thorough monitoring and assessment of the patients' outcomes and recovery following the surgical intervention.

Until the patient's post-operative visit at 2 weeks (Suture Removal), no brushing or flossing of the surgical defect site was performed. During this visit, the wound healing process was assessed by the investigator, and outer sutures were removed if appropriate. Subsequently, as determined by the investigator, the patient was allowed to resume manual tooth brushing of the treated areas using a soft toothbrush and a careful roll technique.

To promote oral hygiene, all patients were instructed to rinse with warm saltwater mouth rinse twice daily until the 2-week postoperative visit. Direct mastication on the defect site was prohibited for 2 to 4 weeks or until the sutures were removed. Among the participants, three individuals underwent suture removal at the 2-week follow-up appointment, while the remaining three underwent suture removal at the 4-week appointment.

Patients returned for reinforcement of plaque control instructions, evaluation of clinical parameters, monitoring of wound healing, assessment of infection, changes in concomitant medications, and reporting adverse events. Oral hygiene instructions (OHI) and professional cleaning were performed as needed, based on the presence of visible plaque.

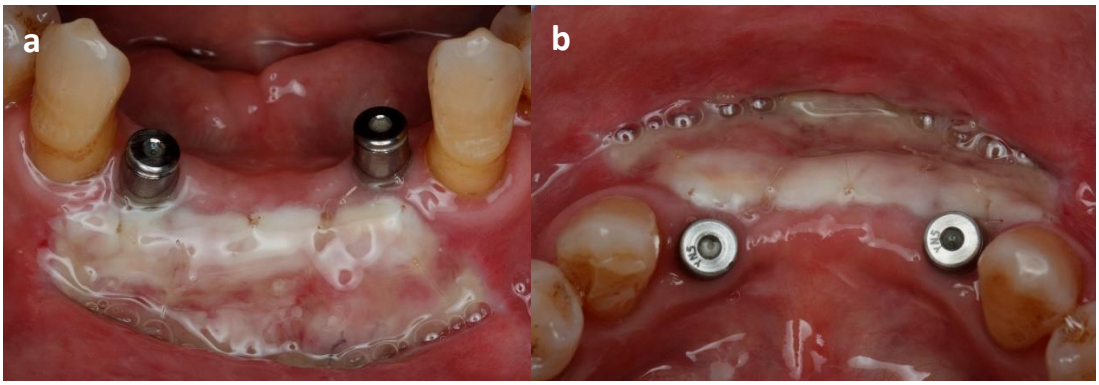

Figure 6. Follow-up at 2 weeks, loose sutures were removed, some sutures were left to resorb. (a) Frontal view. (b) Occlusal view.

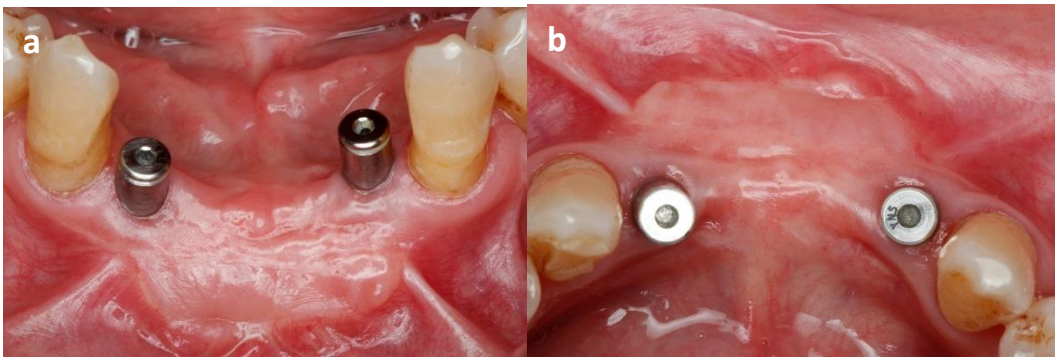

Figure 7. Follow-up at 3-months. (a) Frontal view. (b) Occlusal view.

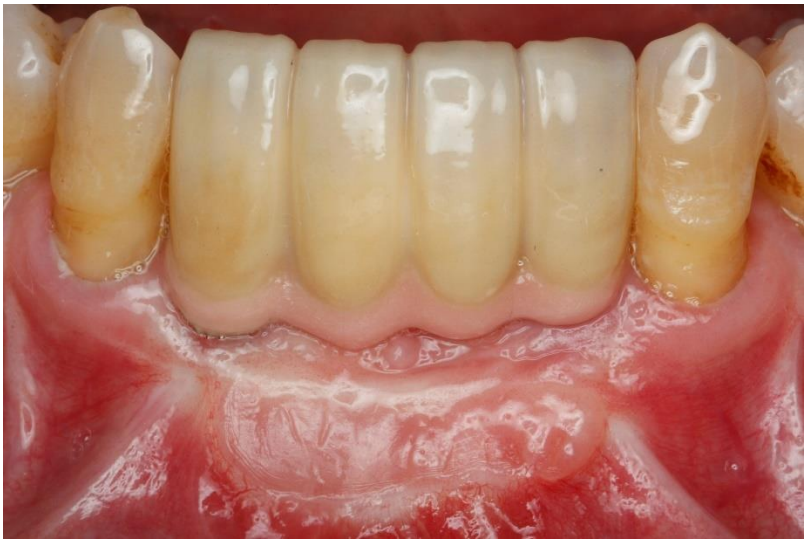

Figure 8. Follow-up at 6-months, Frontal view.

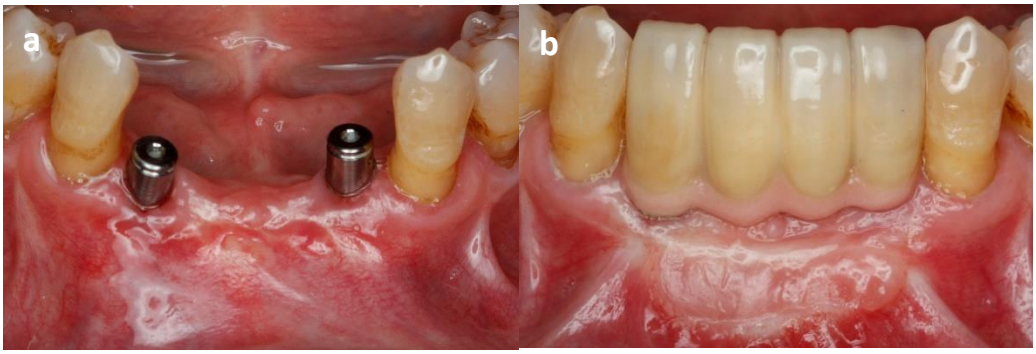

Figure 9. Before and after deepening the vestibule and increasing KT. (a) Pre-op photo. (b) 6-months post-op.

Patient-centered outcomes

All patients were instructed to complete a customized visual analogue scale (VAS) (Figure 7) to assess pain, swelling, bruising, and impact on daily activity. This scale, ranging from 1 to 10, was utilized from the day following the surgical visit until the 2-week follow-up appointment. A score of 1 indicated minimal pain/swelling/bruising/impact on daily activity/esthetic satisfaction, while a score of 10 represented the highest level of pain/swelling/impact on daily activity.

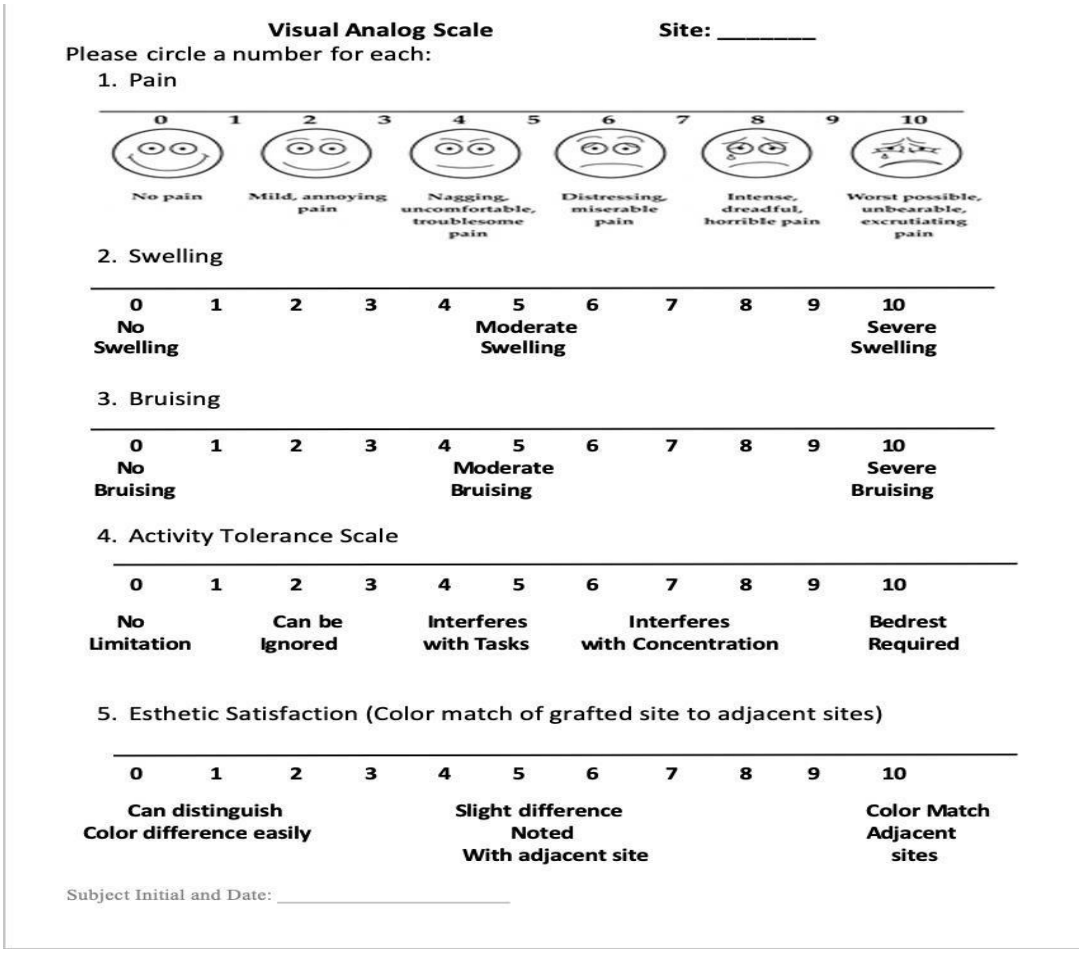

Figure 10. Visual Analog Scale

Practitioner-Determined Esthetic

The evaluation of esthetics, based on the Pink Esthetic Score (PES) criteria (Table 4), was conducted by the calibrated examiner Dr. Hussein Basma during the screening visit and will be conducted at the 6-month follow-up visit for each surgical site. The PES assessments will be documented at the conclusion of the study, the 12-month follow-up appointment as well.

Variables	0	1	2
Tissue contours	Unnatural	Virtually natural	Natural
Gingival level	>2 mm	1–2 mm	<1 mm
Alveolar process	Clearly resorbed	Slightly resorbed	No difference
Coloring	Clear difference	Slight difference	No difference
Texture	Clear difference	Slight difference	No difference

Table 4. Pink Esthetic Score (PES).

Novel Digital Tool to Measure Soft Tissue Difference

In addition to the clinical assessment of pre-operative and post-operative soft tissue augmentation, a novel digital approach was employed to quantify the extent of soft tissue gain (tissue thickness) following the procedure. This methodology involved utilizing intraoral scans (TRIOS ®, 3Shape, Copenhagen, Denmark) to measure the soft tissue thickness changes.

To achieve accurate superimpositions, Geomagic ® Control X ™ (3D Systems, Inc., South Carolina, USA) was utilized. The intraoral scan data, stored as standard stereolithography (STL) files, were converted into polygonal data consisting of numerous triangular data points that reconstructed the digital image. Alignment of the pre-operative and post-operative intraoral scans was achieved by using unchanged reference points, such as teeth. The pre-operative intraoral scan served as the reference model, with segmentation employed to combine the polygons representing the teeth into a unified region. The post-operative intraoral scan (taken at three and six months) was then

imported as the measured model, and alignment was performed through Initial Alignment followed by Best Fit Alignment, utilizing the designated teeth as reference data for alignment purposes (Figure 11). This enabled us to generate measurements of the gained soft tissue thickness (Initial vs 3-months). Moreover, two patients in this study underwent a similar evaluation at the 6-month mark, comparing the changes in tissue thickness by superimposing the 3-month and 6-month intraoral scan. The remaining participants will undergo the same measurement protocol during their respective appointments as scheduled.

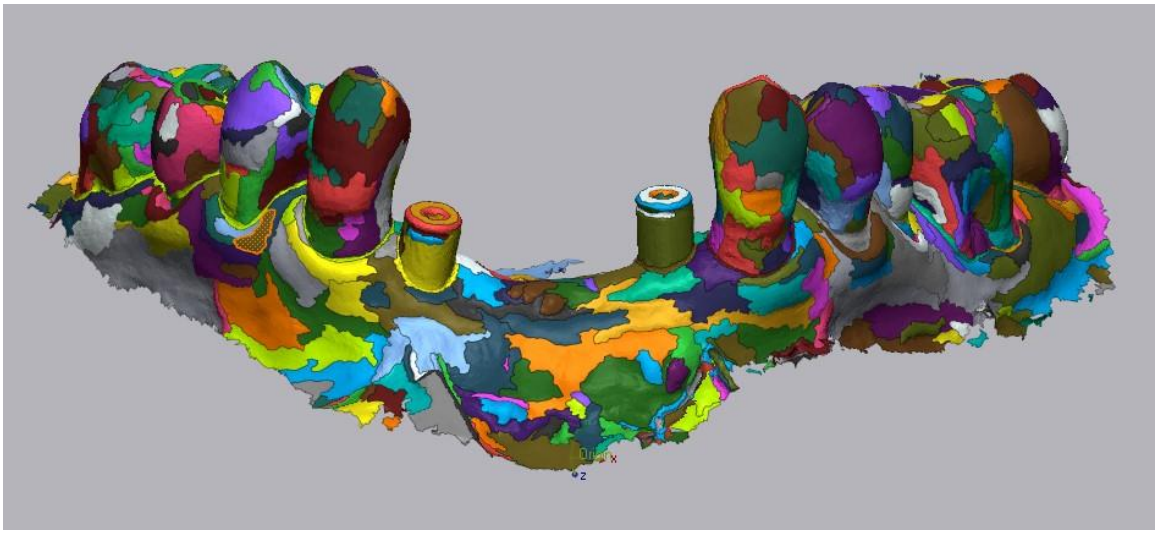

Figure 11. Pre-operative and post-operative intraoral scans superimposed after using Initial Alignment and Best Fit Alignment using only the teeth as reference data for alignment. (Initial and 3-months intraoral scans).

To enable two-dimensional comparisons, two distinct planes were generated at the augmented site, as depicted in Figure 12.

1. A plane located 2 mm from the mucosal margin.
2. A plane positioned 4 mm from the mucosal margin.

The 2D Comparison Tool was employed to obtain average linear measurements of soft tissue thickness within each of these planes (Figure 13). The mesio-distal extension was

determined by assessing the gap between adjacent natural teeth, while the apico-coronal and bucco-lingual extension was defined by the midpoint positioned 2 mm away from the Mucosal Margin and the point located 4 mm apical from the buccal aspect of the Mucosal Margin.

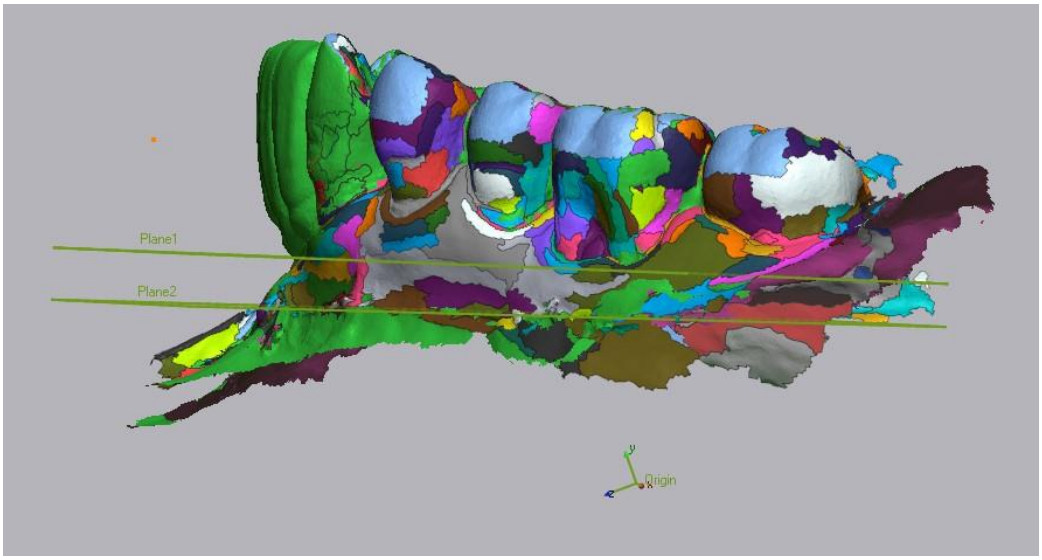

Figure 12. Orthogonal view of both planes.

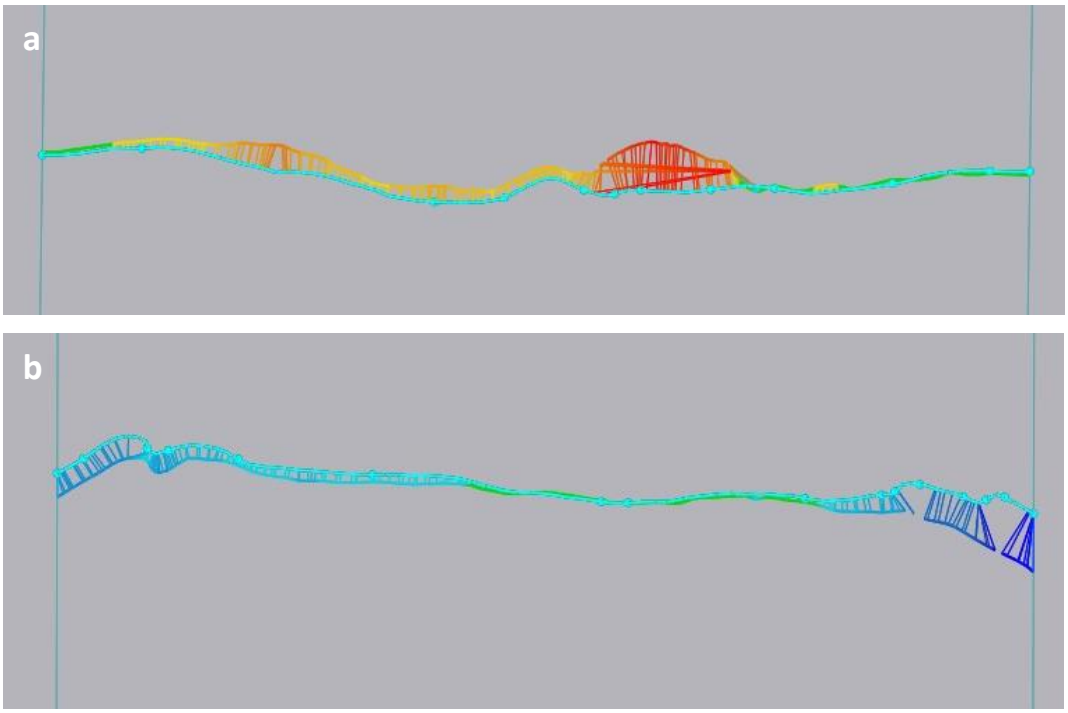

Figure 13. (a) 2D Comparison of pre-operative and post-operative intraoral scans 2 mm from the Mucosal Margin (TT1). (b) 2D Comparison of pre-operative and post-operative intraoral scans 4 mm from the Mucosal Margin (TT2). Red color represents “gain” and blue represents “loss”.

Comparison of the 3-month and the 6-month intraoral scans enabled us to generate linear measurements to assess for soft tissue thickness changes, as shown below in figure 14.

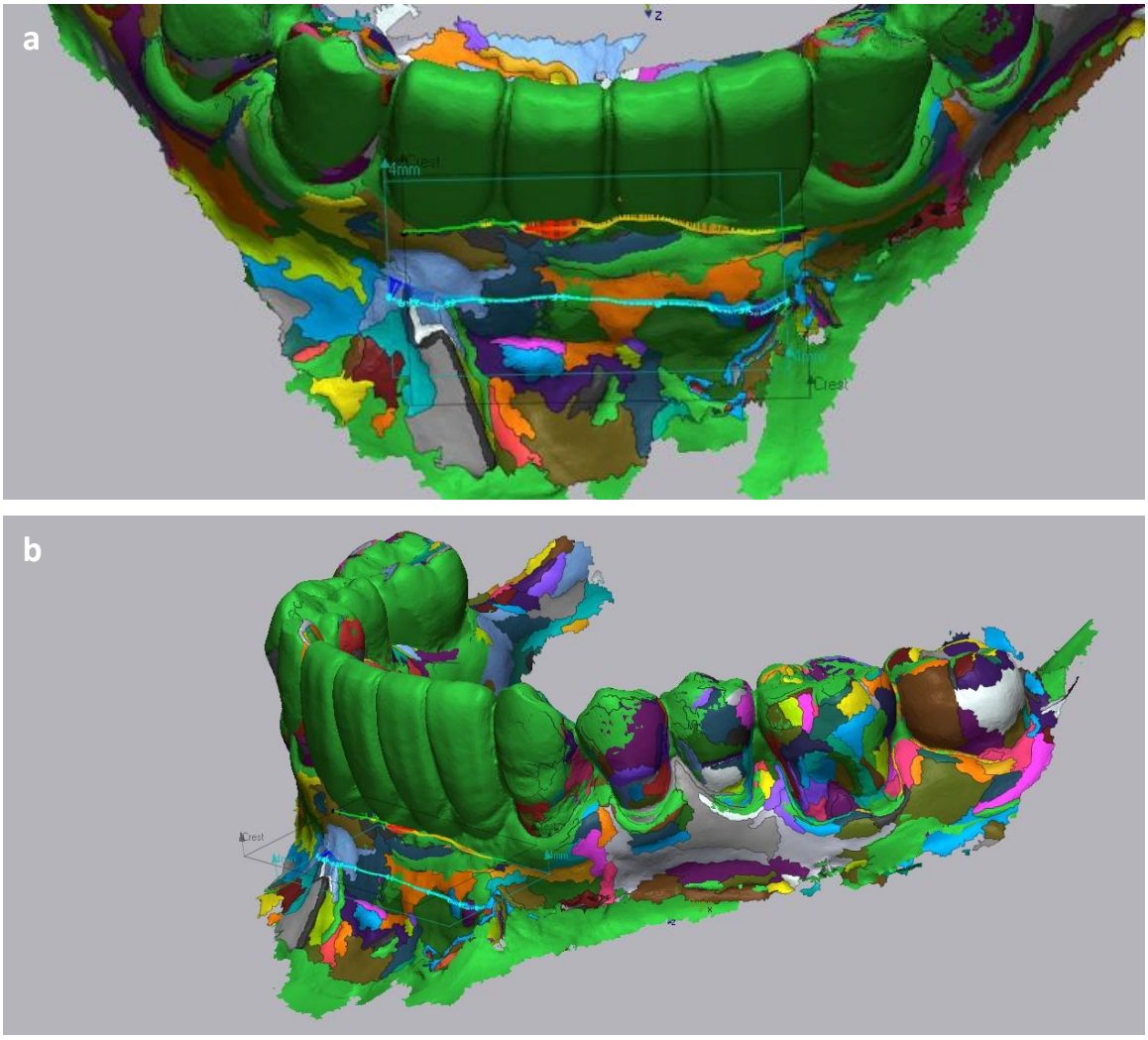

Figure 14. 2D Comparisons along both planes superimposing the 3-month and the 6-month intraoral scans. (a) Orthogonal view of both planes. (b) Planes are visible.

Statistical Analysis

Before the surgical procedure, a calibrated faculty member recorded clinical measurements using a manual periodontal probe (UNC-15) with accuracy to the nearest millimeter. These measurements were subsequently repeated at 3 and 6 months. The measurements will also be taken at the 12-month follow-up appointment. Descriptive statistics were employed, calculating mean and standard deviation (SD) for continuous variables, and frequency and percentage for categorical variables. Statistical significance was determined using a two-tailed test, with a p-value of < 0.05 considered significant.

The statistical analysis focused on assessing the impact of surgical procedures on clinical parameters, primarily evaluating changes from baseline to 3 months, and extending up to 6 months for two patients. Comparative analysis of dental indices, including probing depths, recession, attached tissue, keratinized tissue, TT1, and TT2, was conducted using paired t-test.

RESULTS

The study population comprised six patients who were enrolled from August 2022 to May 2023. All patients successfully completed the 3-month follow-up appointment, while two patients also completed the 6-month follow-up. Consequently, the primary outcome measure, which evaluated keratinized tissue width (KTW), was assessed at all eight grafted implant sites during the 3-month evaluation appointment. The mean age of the patients was 66.2 years, ranging from 60 to 74, with an equal distribution of three men and three women. A total of eight implant sites were treated across the six patients.

All measurements were taken before grafting, at the surgical visit, except for intraoral soft tissue scanning using TRIOS® (3Shape, Copenhagen, Denmark), which was performed during the screening visit to measure soft tissue thickness. All surgical procedures were conducted on the facial aspect of the anterior mandible, none of the patients had any postoperative complication and healing was uneventful. No complaints of neurosensory disturbances were recorded.

At 7 days, the external surface of the ADM was covered with a whitish tissue layer that was firmly attached to the recipient site along with partial sloughing of the epithelium of the strip graft. At 14 days, the newly formed tissue was red with localized areas of the whitish layer still present. After 30 days, tissue surfaces were more uniform without any signs of residual ADM. Complete soft tissue healing with good match in color with the neighboring tissue along with gain in the vestibular depth and width of keratinized tissue was identified at 3 months follow-up.

The plaque index (PI) was recorded prior to grafting, at three months (for all patients), and at six months (for two patients). Prior to grafting, 37.5% of implant sites had a plaque index of 1, while 62.5% had a plaque index of 0. At the three-month evaluation, after multiple reviews of oral hygiene instructions, 91.7% of sites achieved a

plaque index of 0, while 8.3% had a plaque index of 1 (Table 4). At the six-month evaluation, the three implant sites in the two patients who completed that follow-up appointment had a plaque index of 0.

Table 5. Comparison of Plaque index (PI, Silness & Loe) at baseline vs the 3-month appointment.

	Plaque index (PI)	SGG+ADM (24 sites)	p-value
Prior to grafting	0	62.5% of sites	>0.05
	1	37.5% of sites	
3-month follow-up	0	91.7% of sites	
	1	8.3% of sites	

The gingival index (GI) was assessed prior to grafting, at three months, and at six months. Before grafting, 87.5% of sites had a gingival index of 1, while 12.5% had a gingival index of 0. At three months, the gingival index was recorded as 1 in 45.8% of sites and 0 in 54.2% of sites.

Table 6. Comparison of Gingival Index (GI, Loe and Silness) at baseline vs the 3-month appointment.

	Gingival Index (GI)	SGG+ADM (24 sites)	p-value
Prior to grafting	0	12.5% of sites	>0.05
	1	87.5% of sites	
3-month follow-up	0	54.2% of sites	
	1	45.8% of sites	

Probing depth (PD) was measured at the pre-surgery appointment, three months postoperatively, and six months postoperatively. The mean probing depth prior to grafting was 2.25 mm, which remained consistent at 2.2 mm during the three-month evaluation. This aligns with our procedure, as no significant change in probing depth was expected with this type of gingival augmentation. The same reading was recorded for the two patients examined at the six-month postoperative appointment.

Recession (R) was measured prior to grafting, at three months, and at six months postoperatively. The mean recession prior to grafting was 0.04 mm, with only one site out of the 24 treated sites exhibiting 1 mm of midfacial recession. At both the three-month and six-month evaluations, no recession defects were recorded, indicating that the 1 mm of recession observed pre-surgically resolved to 0 mm by the three-month evaluation.

Keratinized tissue width (KTW) was measured prior to grafting, at three months postoperatively, and at six months for the two patients who completed the six-month evaluation. All treated sites exhibited a significant gain in KTW at three months, with an average width of 5.00 mm (SD 0.67 mm, $P<0.05$). The pre-surgical measurements of KTW had an average of 0.96 mm (SD 0.15 mm). For the two patients who attended the six-month evaluation, their average KTW prior to grafting was 1.2 mm (SD 1.03 mm). At three months, these patients showed a further increase in KTW, with an average of 5.89 mm (SD 1.23 mm). By the six-month evaluation, the average KTW was 5.3 mm (SD 0.62 mm).

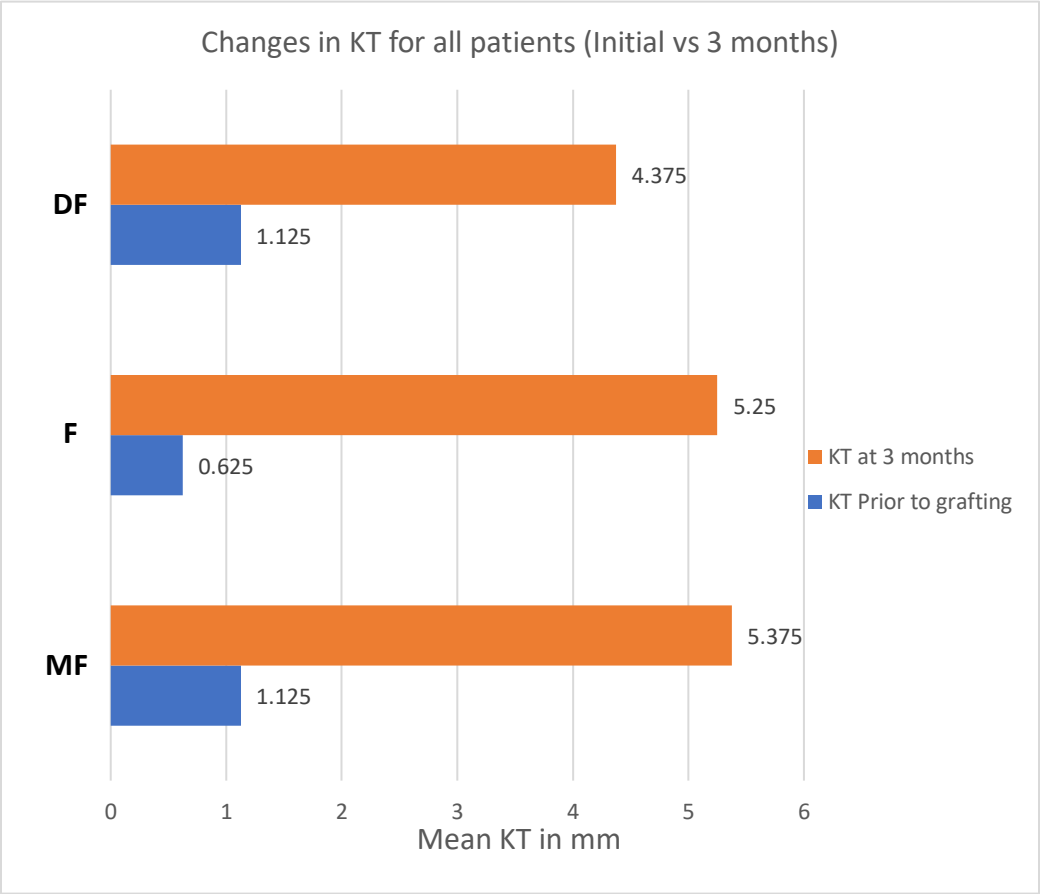

Figure 15. Mean Keratinized tissue (KT) prior to grafting vs 3-months for all patients per site.

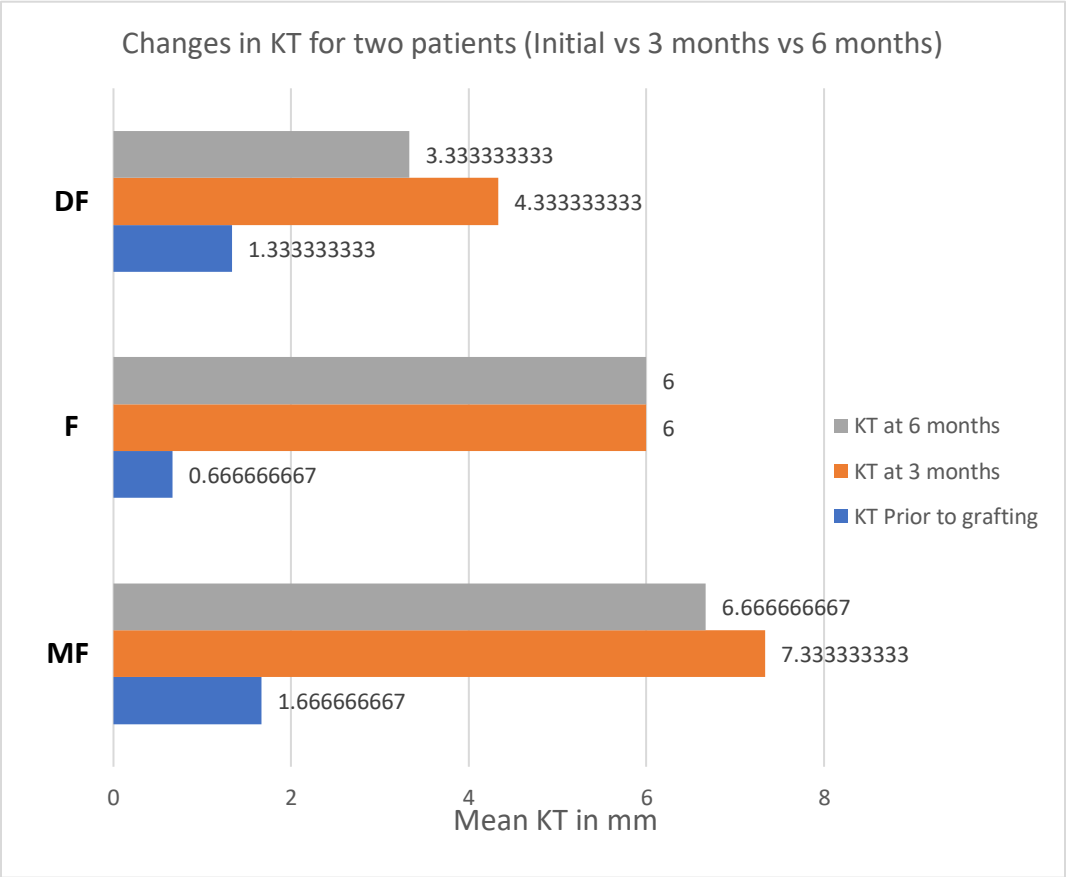

Figure 16. Mean Keratinized tissue (KT) prior to grafting vs 3-months vs 6-months for two patients per site.

Table 7. Mean WKT for each individual subject.

Mean Width of Keratinized Tissue for All Sites Per Patient/mm			
Patient #	Presurgery	3 months	6 months
1	0.67 (mm)	7.33 (mm)	6.00 (mm)
2	2.33 (mm)	3.00 (mm)	4.00 (mm)
3	0.60 (mm)	4.33 (mm)	
4	0.00 (mm)	4.33 (mm)	
5	0.00 (mm)	5.00 (mm)	
6	1.00 (mm)	4.43 (mm)	

Attached tissue (AT) was measured prior to grafting, at three months postoperatively, and at six months for the two patients who completed the six-month evaluation. Prior to grafting, the average attached tissue measurement was 0.34 mm (SD 0.16 mm). At the three-month follow-up appointment, a significant increase was observed, with the average attached tissue measuring 2.79 mm (SD 0.55 mm, $P<0.05$) (Figure 10). Among the two patients who reached the six-month follow-up appointment, the measurements were 0.78 mm (SD 0.92 mm) prior to grafting, 3.44 mm (SD 2.27 mm) at three months, and at six months, the mean attached tissue measured 3.11 mm (SD 0.50 mm) as shown in figure 11.

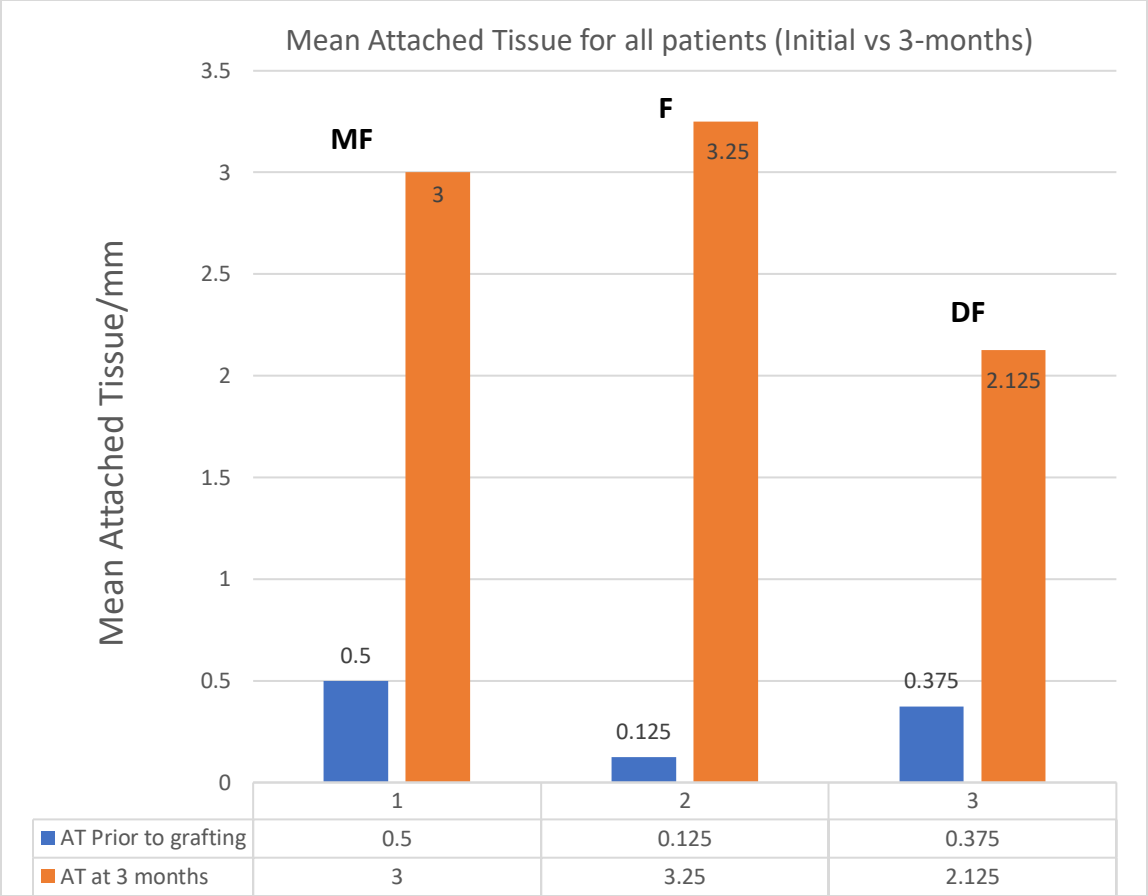

Figure 17. Mean Attached tissue (AT) prior to grafting vs 3-months for all patients per site.

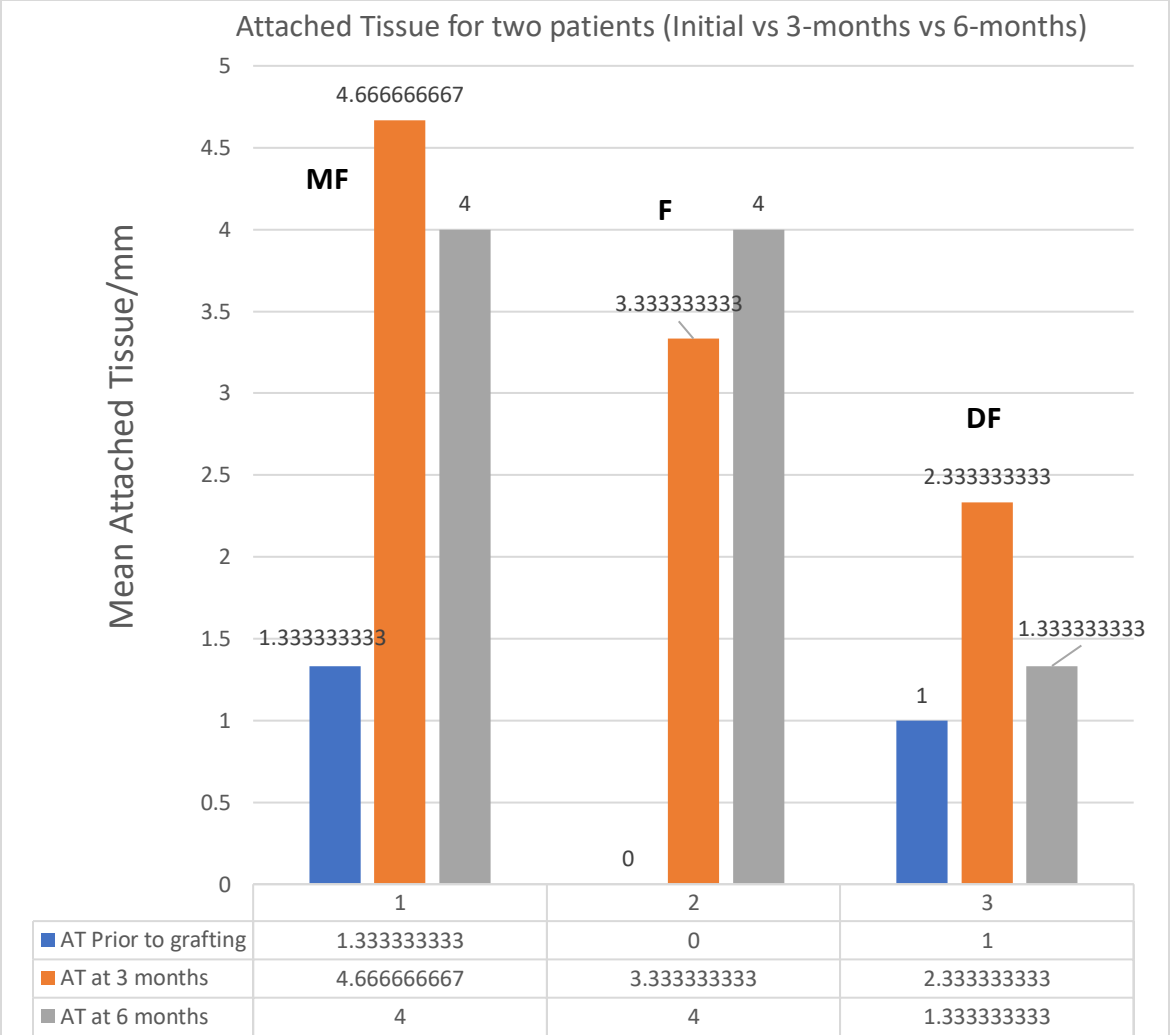

Figure 18. Mean Attached tissue (AT) prior to grafting vs 3-months vs 6-months for two patients per site.

Table 8. Mean Width of Attached Tissue for each individual subject.

Mean Width of Attached Tissue (AT) for All Sites Per Patient/mm			
Patient #	Presurgery	3 months	6 months
1	1.00 (mm)	4.67 (mm)	3.67 (mm)
2	0.33 (mm)	1.00 (mm)	2.00 (mm)
3	0.00 (mm)	2.67 (mm)	
4	0.00 (mm)	2.00 (mm)	
5	0.33 (mm)	2.33 (mm)	
6	0.00 (mm)	2.50 (mm)	

The mean tissue thickness, 2 mm apical to the gingival margin (TT1), was measured pre-surgery and at the three-month evaluation. Prior to grafting, the average TT1 was 2.63 mm, which increased to 3.69 mm at the three-month evaluation (Figure 12). This indicates a significant gain of 1.06 mm in tissue thickness 2 mm apical to the gingival margin ($P<0.05$).

Similarly, the mean tissue thickness, 4 mm apical to the gingival margin (TT2), was assessed pre-surgery and at the three-month evaluation. The average TT2 prior to grafting was 3.5 mm, which increased to 4 mm at the three-month evaluation (Figure 13), representing a gain of 0.5 mm in tissue thickness that was not statistically significant ($P>0.05$).

For the two patients who reached the six-month evaluation, the average pre-surgical TT1 was 2.33 mm. At three months, it decreased slightly to 2.17 mm, but by the six-month evaluation, it increased to 3 mm. Regarding TT2, the average pre-surgical measurement

for these two patients was 2.67 mm. At three months, it increased to 3.33 mm, and at six months, it further increased to 4 mm.

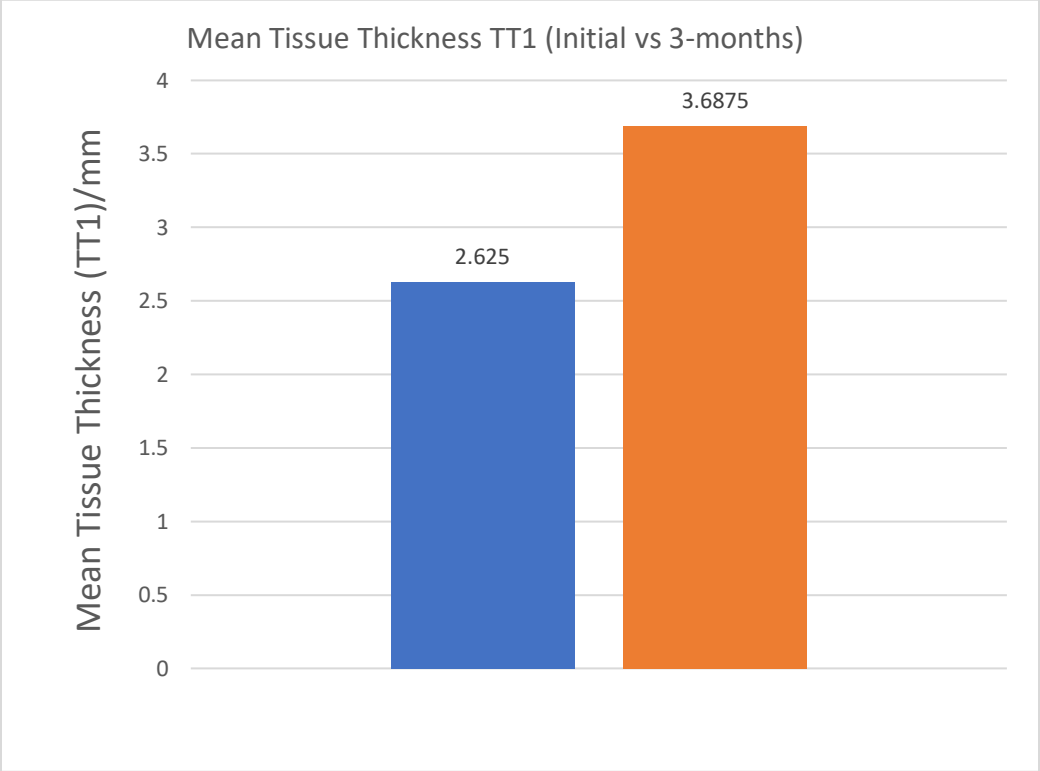

Figure 19. Mean Tissue Thickness (TT1, at 2 mm from the Mucosal Margin).

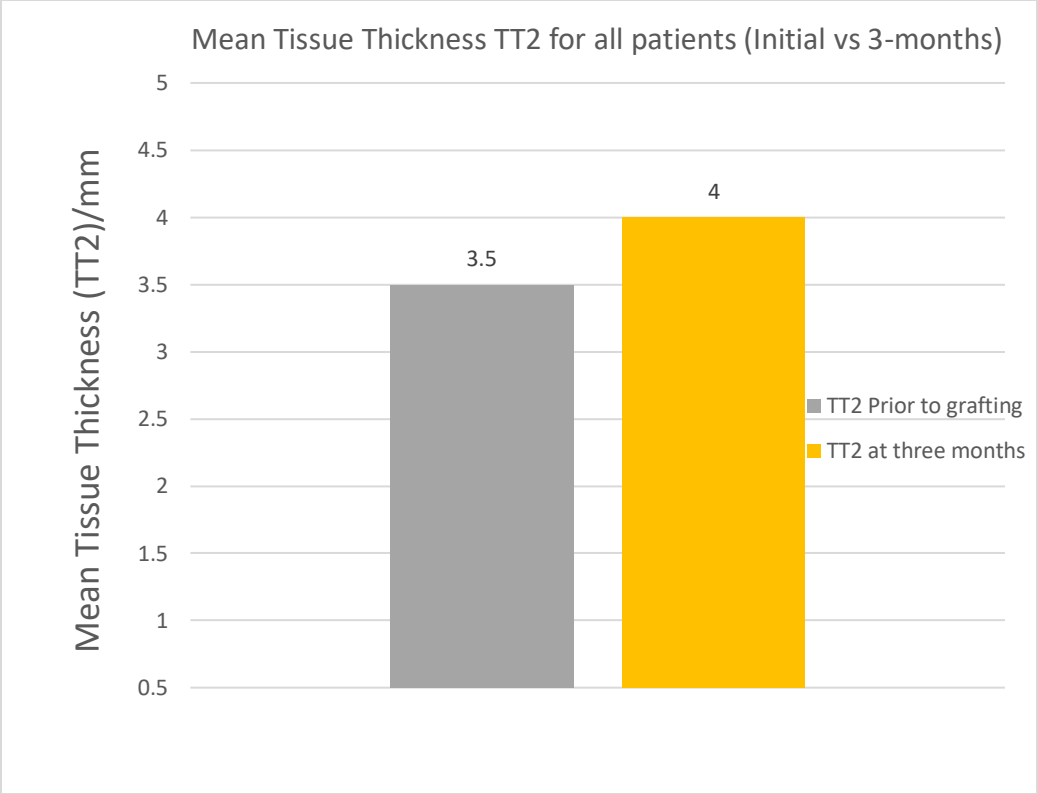

Figure 20. Comparison of Mean Tissue Thickness (TT2, at 4 mm from Mucosal Margin) prior to grafting vs 3-months.

The measurements taken from comparisons of the superimpositions of pre-operative and post-operative intraoral scans are shown in Table 9. These measurements represent changes/gain in soft tissue thickness at TT1 and TT2 (Initial vs 3-months). The mean tissue thickness gain 2 mm from the Mucosal Margin (TT1) was 1.13 mm (SD, 0.90 mm) and a gain of 0.99 mm (SD, 0.74 mm) at 4 mm from the Mucosal Margin.

Table 9. Measurements from superimpositions of intraoral scans.

Patient	TT1 Initial vs 3-months (mm)	TT1 SD	TT2 Initial vs 3-months (mm)	TT2 SD
#1	1.2544	0.9747	1.8113	1.1445
#2	1.1332	0.8214	0.7812	0.5467
#3	1.3455	1.0871	1.0542	1.1333
#4	0.9378	0.7345	0.8224	0.6425
#5	1.0121	0.9273	0.7745	0.4885
#6	1.1137	0.8755	0.6778	0.4779
Mean	**1.1327**	**0.9034**	**0.9869**	**0.7389**

Overall, these results demonstrate positive outcomes in terms of keratinized tissue width, attached tissue, and tissue thickness following the grafting procedure. The significant gains in KTW and tissue thickness indicate successful gingival augmentation. These findings contribute to our understanding of the outcomes and clinical implications of the grafting procedure in enhancing gingival health and tissue quality in the anterior mandible.

Patient-based perception of pain, swelling, bruising, and activity tolerance scale (ATS) were evaluated using the Visual Analog Scale (VAS) (Figure 7) from the day following the surgical visit until the 2-week follow-up appointment. All patients participated in completing the VAS, with data recorded on a scale ranging from 1 to 10, where 1 represented the least severe and 10 denoted the most severe. Findings from the two-week assessment revealed that patients reported a mean pain value of 1.04±0.82, swelling of 0.59±0.74, bruising of 0.23±0.30, and ATS of 0.81±0.72 (Table 9). The mean pain values for all patients for each day are shown in figure 14.

Table 10. Mean VAS Values for all variables.

Variable	***VAS Values (Mean ± SD)***
Pain	1.04±0.82
Swelling	0.59±0.74
Bruising	0.23±0.30
Activity Tolerance Scale (ATS)	0.81±0.72

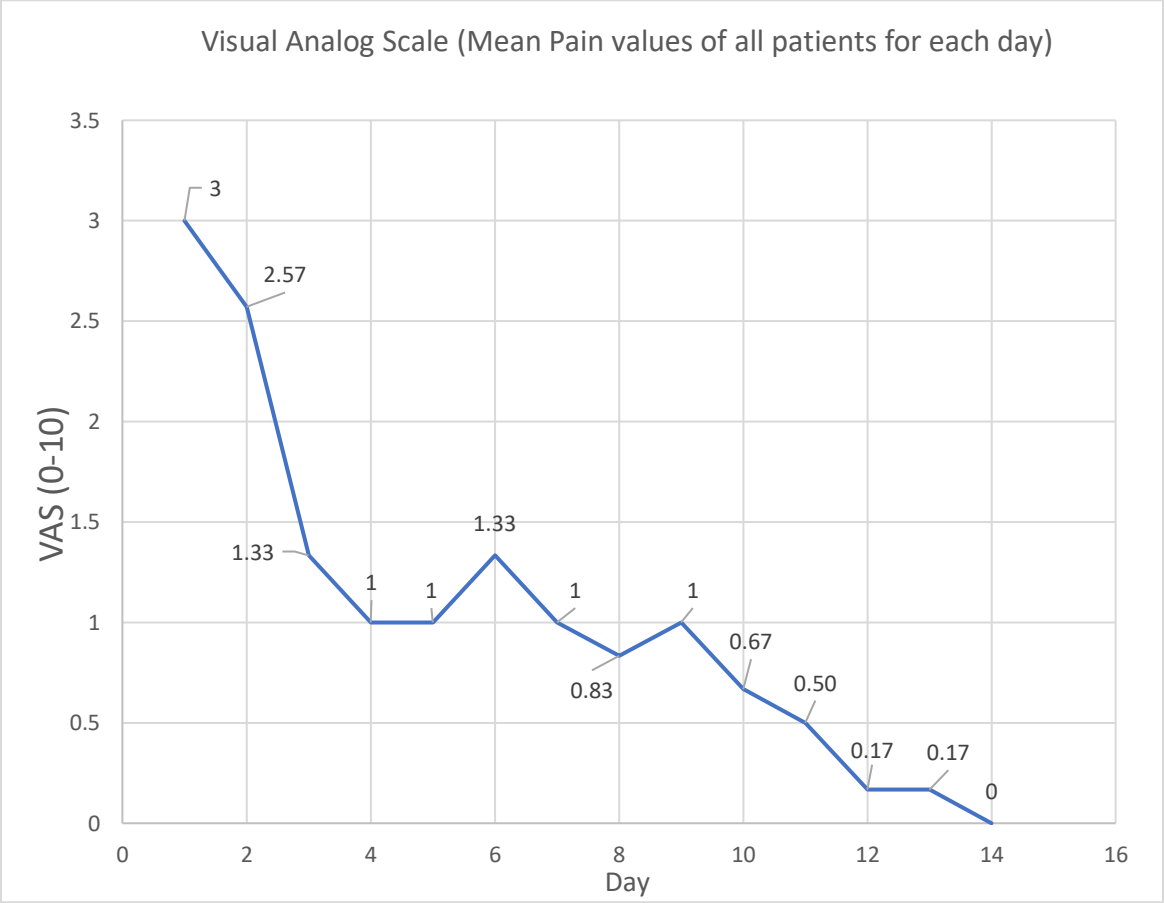

Figure 21. Mean VAS pain values for all patients per day.

DISCUSSION

In the present study, a combination of a strip graft and acellular dermal matrix was used to treat mucogingival defects around implants. These defects, which were in the form of loss of vestibule and keratinized mucosa, resulted mainly from tooth loss and advanced horizontal bone augmentation.

It is believed that the characteristics of the peri-implant soft tissue allow disease to spread faster around implants than teeth, due to longer junctional epithelium, parallel orientation of connective tissue fibers and reduced vascularity (Sculean et al., 2014; Atusta et al., 2016).

The Osteology Foundation Consensus report concluded that adequate peri-implant KM is associated with greater marginal bone stability and better reduction in Plaque and Gingival Indices (Giannobile et al., 2018). Previous clinical studies have indicated that an increased zone of keratinized tissue could effectively maintain the stability of both soft and hard tissues surrounding dental implants, thereby promoting their long-term maintenance (Bouri et al., 2008; Kim et al., 2009). In addition, other advantages have been documented, which encompass a reduction in inflammation, increased resistance against bacterial activity, enhanced esthetics, improved stability of the soft tissue, and improved comfort during oral hygiene routines (Bouri et al., 2008).

Several materials including free gingival grafts (Thoma et al., 2018), connective tissue grafts (Tonetti et al., 2018) platelet rich fibrin (Hehn et al., 2016), and allogenic and xenogeneic grafts (Park, 2006; Thoma et al., 2016) have been successfully reported to augment peri-implant mucosal thickness. Increasing the thickness of the soft tissue offers several benefits, including reducing soft tissue discoloration and visibility of the implant or abutment in patients with a thin tissue phenotype. Thicker tissue also provides restorative dentists with greater volume to create more ideal crown contours, leading to

improved aesthetics and biological advantages. In cases where the soft tissue phenotype is thin, ridge lapping is often required, which limits accessibility for cleaning and lacks long-term esthetic stability. By increasing the patient's soft tissue thickness, the need for ridge-lapping of crown restorations can be minimized, allowing for the development of a more aesthetically pleasing and biologically stable crown emergence profile. This, in turn, facilitates oral hygiene and promotes tissue health for the patient (Lin et al., 2019).

ADM is one of the allogenic alternatives that was introduced in 1996 and was used in several applications including soft tissue augmentation, keratinized tissue augmentation, barrier membrane and for root coverage procedures (Gapski et al., 2005). In this study an adequate width of keratinized tissue (4 ± 0.67mm) was achieved using a combination of ADM and SGT at 3 months and for the two patients who completed the 6 month follow up evaluation, a mean gain of 4.1 mm of KTW was achieved.

Park et al. in 2006, investigated the use of ADM to increase the width of KM and found a mean gain in KM of 2.2 mm after 6 months. Several clinical studies compared the outcomes of ADM compared with the results achieved by FGG (Wei et al., 2000; Agarwal et al., 2015), where they reported a gain of (2.59 mm for ADM; 5.57mm for FGG) and (2.13 mm for ADM; 4.8 mm for FGG) at 12 months respectively. Although all these reported outcomes of gain of KM seems minimal but achieves the goal of having adequate band of keratinized tissue around teeth and implants. In all those studies, ADM was left exposed completely to heal after placed on the recipient site periosteum.

Harris et al., 2001, covered the apical portion of the ADM with a flap containing keratinized tissue and had similar outcomes to this study (4 mm gain of KM). The results of Harris et al., 2001 study are contrary to the results of Wei et al., 2000. In the study by Wei et al., 2000, it was observed that the apical portion of the acellular dermal matrix lacked coverage from a flap containing keratinized tissue. Their results indicated that the increase in keratinized tissue was statistically higher with a free gingival graft (5.57 mm) compared to the use of an acellular dermal matrix (2.59 mm).

A recent study showed that if 0.5 to 1mm width of residual KM was apically positioned to the collagen matrix, a gain of 4.81mm of KM (Jiang et al., 2019). This band of Keratinized tissue that was positioned apically ensured more KM gain due to more blood supply and less tissue shrinkage.

Urban et al., 2015, was the first author to introduce the combination of a strip gingival graft technique with a soft tissue alternative. He reported a mean gain of 6.33 mm at 12 months while there was a 43% shrinkage of the grafted site at 6 months. The SGT acts as an apical barrier that prevents the rebound of soft tissue and provides cell sources of keratinized tissue by creating a recipient bed that is bounded on both coronal and apical ends with KM. The ADM acts as a scaffold that allows cell migration from adjacent tissue (Urban et al., 2015; De Resende et al., 2019). This would justify the longer healing period and better blending with adjacent tissue; however, the apical portion has different color and consistency.

In light of the principle advocating the placement of Alloderm within a keratinized tissue environment and the successful combination of a Strip Gingival Graft (SGG) and a xenogenic collagen matrix (XCM) by Urban et al. (2015) to enhance vestibular depth and augment keratinized tissue, the present study aimed to assess the extent of keratinized tissue augmentation achievable in sites with insufficient keratinized tissue and/or shallow vestibules. The objective was to substantiate the hypothesis that the presence of a greater amount of surrounding keratinized tissue would result in increased keratinized tissue dimensions when utilizing a combination of an Alloderm and a Strip Gingival Graft in an apical fashion. Additionally, the study aimed to compare Visual Analog Scale (VAS) pain values between the novel approach and the conventional gold standard technique of Free Gingival Graft (FGG) for keratinized tissue augmentation. By examining these parameters, a comprehensive evaluation of the effectiveness and patient comfort associated with the proposed method could be conducted, contributing to the advancement of clinical practices in augmenting keratinized tissue.

The combination of ADM and SGT was well tolerated by patients with minimal morbidity and analgesics consumed were reported. This could be related to the minimal size of FGG strip harvested from the palate when compared to regular FGG which most of the times leaves a large wound area to heal by secondary intention (Griffin et al., 2006; Tavelli et al., 2019).

The utilization of intra-oral connective tissue (CTG) and epithelialized free gingival grafts (FGG) from the palate as donor sites has been widely practiced. However, the healing process of FGG by secondary intention typically takes approximately 2-4 weeks (Farnoush, 1978), and it has consistently been associated with increased discomfort for patients due to post-operative pain and bleeding (Farnoush, 1978; Jahnke et al., 1993; Del Pizzo et al., 2002). The present study reported a mean Visual Analog Scale (VAS) pain value of 1.04±0.82, with a peak VAS pain value of 3.00 on day 1 and 2.57 on day 2.

In a randomized clinical trial by Femminella et al., 2016, platelet-rich plasma was found to enhance palatal healing and reduce morbidity when compared to gelatin sponge. The study demonstrated a lower mean discomfort VAS score of 2.4±0.88, contrasting with the findings of Zucchelli et al., 2010, where the mean VAS value was 3.1±1.99.

Another investigation by Tavelli et al., 2017, assessed VAS pain values when employing a collagen sponge and cyanoacrylate to cover the palate after harvesting a free gingival graft. The VAS values recorded were consistently below 0.6, with the peak of pain occurring on the 3rd day at 0.58±0.92. The beneficial effect of cyanoacrylate, in combination with an underlying collagen sponge, is thought to arise from its sealing, bacteriostatic, and hemostatic properties, which result in the formation of a protective layer isolating the wound from the oral cavity. Consequently, the comprehensive seal and wound protection provided by cyanoacrylate likely contribute to decreased postoperative morbidity and pain.

Taken together, these findings suggest that alternative approaches such as platelet-rich plasma and cyanoacrylate, when combined with suitable materials, have the potential to improve palatal healing outcomes and minimize postoperative discomfort. The establishment of an effective seal and protection of the surgical wound appear to be crucial factors in reducing morbidity associated with palate donor sites. The mean VAS pain value was comparable to the VAS value in Tavelli et al., 2017 study.

ADM has been successfully proven to repair gingival recession and to increase keratinized tissue (Gapski et al., 2005). However most studies included a high shrinkage percentage for this allograft material. This study added a strip graft that reduced the amount of shrinkage associated with using ADM and created a wider zone of KM.

Despite its advantages of gaining keratinized tissue, pleasing esthetic outcomes and being less traumatic for the patient, ADM excessive shrinkage is one of the drawbacks for using this allograft material. In a recent randomized trial, ADM demonstrated a greater shrinkage than FGG (56% vs 12%) (De Resende et al., 2019). In another study (Cevallos et al., 2019), a 15-year clinical study, reported a 59.6% shrinkage of ADM. This amount of ADM shrinkage was smaller when compared to other studies where they found a shrinkage of 71% and 76.6% (Wei et al., 2000; Agarwal et al., 2015).

A well-designed clinical study with a control group to compare outcomes with the proposed treatment can help in drawing firm conclusions. Another limitation of this study was the short period of evaluation (3-6 months). With most patients being evaluated only up to the 3-month evaluation appointment, a longer follow-up period is essential to assess any further changes.

SUMMARY AND CONCLUSIONS

The present prospective case series aimed to investigate the effectiveness of combining a Strip Gingival Graft (SGG) and Acellular Dermal Matrix (ADM) to augment the width of keratinized tissue (WKT) around dental implants in the lower anterior mandibular area. The primary focus of this study was to assess the increase in WKT, while secondary objectives included evaluating changes in tissue thickness at 2 mm (TT1) and 4 mm (TT2) from the mucosal margin, as well as measuring patient-centered outcomes such as pain, bleeding, bruising, swelling, and the impact on daily activities.

After a three-month postoperative evaluation, no significant changes were observed in the probing depth (PD) and gingival index (GI) when compared to the screening visit. However, there was a significant gain in WKT, with an average width of 5.00 mm (SD 0.67 mm, $P<0.05$). This result indicated that the combination of SGG and ADM effectively increased the width of keratinized tissue around dental implants. Moreover, a significant increase in tissue thickness of 1.06 mm ($P<0.05$) was observed at 2 mm apical to the gingival margin (TT1). Although there was a 0.5 mm gain in tissue thickness at 4 mm apical to the gingival margin (TT2), the difference was not statistically significant ($P>0.05$).

For the subset of patients who attended the six-month evaluation, their average initial KTW prior to grafting was 1.2 mm (SD 1.03 mm). At the three-month evaluation, these patients demonstrated further improvement in KTW, with an average of 5.89 mm (SD 1.23 mm). By the six-month evaluation, the average KTW was 5.3 mm (SD 0.62 mm). These findings indicate a sustained increase in WKT over time, highlighting the effectiveness of the SGG and ADM combination in achieving a wide band of Keratinized Tissue (KT).

The results of this study provide valuable insights into the clinical outcomes of utilizing SGG and ADM for WKT augmentation around dental implants. The significant increase in

WKT and tissue thickness observed at the three-month evaluation demonstrates the effectiveness of this combined technique. The wider width of keratinized tissue offers several advantages, including improved esthetics, reduced soft tissue complications, and enhanced oral hygiene maintenance.

In terms of patient-centered outcomes, this study also assessed pain, bleeding, bruising, swelling, and effects on daily activities. It is worth noting that the study reported minimal postoperative discomfort based on the visual analog scale (VAS) pain values, which averaged 1.04±0.82. This suggests that the utilization of an SGG minimizes trauma to the donor sites, potentially attributed to the rapid epithelialization facilitated by close wound edges and the standardized SGG width of 3 mm.

In conclusion, the findings of this prospective case series support the use of a combination of SGG and ADM for augmenting the width of keratinized tissue around dental implants in the lower anterior mandibular area. The technique demonstrated a significant increase in WKT and tissue thickness, while maintaining favorable periodontal parameters.

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APPENDIX A

IRB Approval Letter

THE UNIVERSITY OF ALABAMA AT BIRMINGHAM
Office of the Institutional Review Board for Human Use

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701 20th Street South
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APPROVAL LETTER

TO: Basma, Hussein S

FROM: University of Alabama at Birmingham Institutional Review Board
Federalwide Assurance # FWA00005960
IORG Registration # IRB00000196 (IRB 01)
IORG Registration # IRB00000726 (IRB 02)
IORG Registration # IRB00012550 (IRB 03)

DATE: 10-Oct-2022

RE: IRB-300006910
IRB-300006910-006
Gain of Keratinized Mucosa Around Dental Implants Using a Combination of Strip Gingival Graft and Acellular Dermal Matrix

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The IRB reviewed and approved the Revision/Amendment submitted on 19-Sep-2022 for the above referenced project. The review was conducted in accordance with UAB's Assurance of Compliance approved by the Department of Health and Human Services.

Type of Review: Expedited
Expedited Categories: b2
Determination: Approved
Approval Date: 10-Oct-2022
Expiration Date: 31-May-2023

The following apply to this project related to informed consent and/or assent:

- Waiver (Partial) of HIPAA

Documents Included in Review:

- IRB EPORTFOLIO

To access stamped consent/assent forms (full and expedited protocols only) and/or other approved documents:
1. Open your protocol in IRAP.
2. On the Submissions page, open the submission corresponding to this approval letter. NOTE: The Determination for the submission will be "Approved."
3. In the list of documents, select and download the desired approved documents. The stamped consent/assent form(s) will be listed with a category of Consent/Assent Document (CF, AF, Info Sheet, Phone Script, etc.)