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Comparison of two different surgical approaches to increase peri-implant mucosa thickness: a randomized controlled clinical trial

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COMPARISON OF TWO DIFFERENT SURGICAL APPROACHES TO INCREASE PERI-IMPLANT MUCOSA THICKNESS:

A RANDOMIZED CONTROLLED CLINICAL TRIAL

by

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CERTIFICATE OF APPROVAL

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MASTER'S THESIS

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ABSTRACT

Objectives: Tooth replacement therapy using endosseous implants has become an essential component of contemporary dental practice. While a plethora of factors determine clinical success, the bucco-lingual and apico-coronal dimensions of the peri-implant mucosa play an important role in both esthetics and the maintenance of peri-implant health. Studies, most of which treat mucogingival defects in the natural dentition, comparing acellular dermal matrix (ADM) and autologous subepithelial connective tissue grafts (sCTG) have shown similar clinical outcomes. The purpose of this non-inferiority trial is to determine the clinical efficacy of ADM in the augmentation of peri-implant mucosa thickness (PMT) as compared to an autologous sCTG in human adults.

Methods: Twenty healthy adults treatment planned for a single tooth implant restoration in need of simultaneous peri-implant mucosa augmentation at the time of implant placement were recruited on the basis of an eligibility criteria. Patients were randomly assigned to the control group (autologous sCTG), or the experimental group (ADM allograft). Clinical measurements of mucosal thickness at the site were made with a periodontal probe and an endodontic spreader at baseline and 16 weeks post-op. These measurements were made by a masked, calibrated examiner. Gingival health, oral hygiene, wound healing and patient reported outcomes were also obtained. Mann-Whitney U tests were used to compare the mean mucosal thickness changes between the groups.

Results: The mean gain in PMT was approximately 1.5mm in the control group and 0.8mm in the experimental group. When measured at 1, 3 and 5mm apical from the CEJ, only the 3mm site exhibited a difference between the groups that approached statistical significance (control: 2.08 ± 0.80mm, test: 0.83 ± 1.37mm, Mann Whitney U = 10.00, p=0.05). Changes in keratinized mucosa width, healing index and patient reported outcomes were generally similar between the two groups.

Conclusions: Within the limitations of this study, both autologous sCTG and ADM appear to be adequate materials to augment PMT without sacrificing other relevant clinical parameters and/or patient related outcomes.

PUBLIC ABSTRACT

Tooth replacement using dental implants is a common procedure. Among other factors, the thickness of the gum tissue over the dental implant is important for an esthetic outcome and maintenance of long-term health. In cases of thin tissue, a gum graft can be done at the time of implant placement. Two commonly used grafts are acellular dermal matrix, a commercially available human-derived grafting material, or connective tissue obtained from the patient's palate. While both grafts have distinct technical advantages, current evidence shows similar healing outcomes between the two when treating natural teeth.

The purpose of this study was to determine the clinical efficacy of donor acellular dermal matrix in the augmentation of the thickness of the mucosa over the dental implant as compared to a palatal connective graft. Using data from clinical measurements and patient-reported outcomes, results showed no significant differences between the two. In the light of the results from this study, donor acellular dermal matrix and palatal connective tissue may be used interchangeably to thicken gum tissue over dental implants.

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CHAPTER 1. Introduction

1.1. Periodontal Anatomy

Adjacent to teeth, the oral mucosa is composed of two different tissue types: masticatory mucosa and lining mucosa. The masticatory mucosa covering the coronal portion of the alveolar process and the cervical portion of the clinical dental crown is referred to as the keratinized tissue, or simply gingiva, and spans vertically from the free gingival margin to the mucogingival junction (Lindhe et al., 2008, Zuhr and Hürzeler, 2012). The distance from the free gingival margin to the mucogingival junction is often referred to as keratinized mucosa width, which broadly varies between individuals and within the same individual, depending on the location in the mouth. The widest dimension of gingival width is typically found at the facial of the maxillary incisors, and the narrowest dimension at the buccal of the mandibular premolars and lingual of the mandibular incisors (Zuhr and Hürzeler, 2012). The epithelial surface of the masticatory mucosa is keratinized except in the region of the interdental col, which is non-keratinized (Lindhe et al., 2008).

The lining mucosa lies apical to the masticatory mucosa, except in the hard palate, which is covered by entirely masticatory mucosa. It also lines the floor of the mouth, cheeks, soft palate and ventral tongue. The lining mucosa adjacent to teeth is also known as the alveolar mucosa, as it covers the remaining alveolar ridge apical to the masticatory mucosa. The lining mucosa is mobile, thin, and is not keratinized.

Adjacent to the tooth surface, a sulcus forms as the free gingival margin apexes and rolls back towards the tooth. The sulcus is lined by oral sulcular epithelium, which is nonkeratinized. The outer gingival epithelium is parakeratinized in which remnants of cell nuclei are present in the outermost epithelial layer, or orthokeratinized, which the outer cell layer is lacking nuclei. The depth of the sulcus varies among individuals and is one measure of periodontal health. Continuing apically down the root surface, the soft tissue eventually forms an attachment to the tooth. This attachment consists of an epithelial and connective tissue attachment. At the coronal level of soft tissue attachment to tooth, sulcular epithelium merges with junctional epithelium. The junctional epithelium consists of three to four layers of non-keratinized epithelium and is

attached to the tooth by a basal lamina and to the underlying gingival connective tissue by an external basal lamina. reference Apical to the epithelial attachment, a connective tissue attachment forms on the cementum. The connective tissue attachment to the tooth coronal to the alveolar bone crest functions to unite the teeth to the marginal gingiva (circular fibers, dento-gingival fibers and dento-periosteal fibers) adjacent teeth, and alveolar bone crest (trans-septal fibers) (Lindhe et al., 2008).

1.1.1. *Periodontal Biologic Width*

In a classic study published in 1961, Gargiulo and colleagues described the gingival attachment to the tooth. This biological interface was defined by three distinct topographical zones; the sulcus, the connective tissue fibrous attachment and the epithelial attachment. Using microscopic measurements of sectioned teeth from cadaver jaws, the authors found the mean sulcus depth to be 0.69mm, the length of the epithelial attachment 0.97mm and the length of the connective tissue attachment 1.07mm (Gargiulo et al., 1961). This dentogingival attachment was later coined as the "biologic width" by Cohen in 1962.

In a similar study, Vacek and collaborators provided additional information on the dimensions of the dentogingival junction using block sections of 10 human cadaver jaws. The investigators found the mean sulcus depth to be 1.32±0.80mm, the length of the epithelial attachment 1.14±0.49mm and the length of the connective tissue attachment 0.77±0.29mm. It is interesting to note that there was considerable variation in the connective tissue and epithelial attachment between subjects and within subjects. The range in biologic width was 0.75mm to 4.33mm, and the biologic width was greater in the posterior segments (Vacek et al., 1994).

In a recent systematic review, the mean values of biologic width obtained from meta-analysis ranged from 2.15 – 2.30mm. The authors stressed there is significant variability between individuals and between sites in the same individual. There are many factors that influence the biologic width, therefore a standard reference number that would suit a majority of sites cannot be established (Schmidt et al., 2013).

1.1.2. ***Periodontal Biotype***

While the apico-coronal gingival width has received much attention in periodontology, the bucco-lingual thickness is equally as significant, but the literature on this dimension is less abundant. The so-called gingival biotype, or phenotype, describes the bucco-lingual dimension and morphology of the marginal gingiva. Many attempts have been made to accurately measure and predict the biotype. Ochsenbein and Ross discussed the relationship between the gingival topography and underlying bone, specifically the differences in scalloped architecture and flat architecture. They stated "Tooth form, relationship of tooth to bone, relationship of one tooth to another, and other anatomic factors are responsible for the behavior and form of the normal gingiva" (Ochsenbein and Ross, 1969). Becker and colleagues, using measurements obtained from 111 dry skulls, elaborated the description of bony scallop described by Ochsenbein & Ross. The mean distance from the mid-buccal alveolar crest to the interdental bone crest ranged from 2.1mm in the flat group, 2.8mm in the scalloped group and 4.1mm in the pronounced scallop group (Becker et al., 1997). Interestingly, the authors did not confirm a definitive relationship between tooth shape and bone morphology.

Investigators then began to evaluate the relationship of biotype and the reaction to periodontal disease (i.e. gingival recession or pocket depth formation). Olsson and collaborators confirmed their hypothesis that individuals with long-narrow incisors are more prone to gingival recession than those with short-wide incisors. In fact, while defining the subject's biotype on the crown form of the central incisors only, those in the long-narrow group exhibited more recession on incisors, canines, premolars and molars (Olsson and Lindhe, 1991). While conclusions were stated that individuals with a long-narrow tapered crown have a thinner periodontium that is more susceptible to gingiva recession, no attempt was made to measure the bucco-lingual dimension of the gingiva.

In 1977 Goaslind and colleagues used a novel transformer probe to measure gingival thickness in both free and attached gingiva. The mean thickness at the depth of the gingival sulcus for all teeth measured was 1.56±0.39mm and ranged from 0.53mm to 2.62mm. At the midway point between the depth of the sulcus and the mucogingival junction, the mean thickness was 1.25±0.42mm and ranged from 0.43mm to 2.29mm.

Interestingly, the thickness measured at the depth of the sulcus was directly proportional to the free gingival width, while the thickness at the midpoint from the depth of the sulcus to the mucogingival junction was inversely proportional to the gingival width (Goaslind et al., 1977).

In 2000, Müller used an ultrasonic device to measure the thickness of masticatory mucosa in 40 healthy volunteers. The ultrasonic device had been previously validated for reproducibility to other means of measurement, with measurement error at facial and lingual gingiva of 0.26mm. The facial gingiva was measured at 1-2mm apical to the gingival margin, the mean thickness ranged from 0.70±0.15mm at maxillary canines, 081±0.28mm at maxillary second premolars, 0.84±0.21mm at maxillary first premolars, 0.86±0.33mm at maxillary lateral incisors, and 1.00±0.30mm at maxillary central incisors. The facial gingival thickness was considerably less in mandibular teeth and in females (Muller et al., 2000b).

Müller, in a separate study, also reported the thickness of masticatory mucosa in subjects with different gingival biotypes. Three clusters were formed from the same 40 subjects; thin gingiva, slender tooth form and a narrow band of keratinized tissue (A1), thin gingiva, slender tooth form and a wider band of keratinized tissue (A2) and thicker and wider gingiva with quadratic tooth form, particularly central incisors (B). Individuals in cluster B had the statistically significantly thicker masticatory mucosa and wider gingiva, but differences in recession were not statistically significant. The investigators also found thinner palatal mucosa in those with slender tooth form and a narrow band of keratinized tissue, causing a potential challenge in gingival grafting in a group more at risk for recession (Muller et al., 2000a).

More recently, investigators have measured the bucco-lingual thickness to determine the outcomes and predictability of root coverage procedures. In a case series of nineteen coronally positioned flap procedures, the mean flap thickness was 0.7 ± 0.2mm, overall root coverage was 82 ± 17% and in sites where initial flap thickness was > 0.8mm, 100% root coverage was observed (Baldi et al., 1999). In a systematic review aimed to summarize data from root coverage studies analyzing gingival flap thickness, it was determined a critical flap thickness for successful root coverage may exist, but in analyzing many papers of heterogeneous design, a critical baseline thickness was not identified. The authors noted reported means across studies were used in

statistical analysis, and no controlled trials were identified that specifically evaluate the effect of baseline flap thickness on root coverage (Hwang and Wang, 2006).

While many authors dichotomize the periodontal biotype into thin and thick, it is a rather subjective classification, and many clinical presentations can be classified in between the two extremes. Nonetheless, the periodontal biotype is an extremely important factor in periodontics, as differences in the distance from interproximal contact point to inter-radicular bone crest, height of the papilla, thickness of the facial bone crest, and bucco-lingual dimension of the gingiva are prognostic variables in perio-plastic and implant surgery (Zuhr and Hürzeler, 2012, Chambrone, 2015, Lee et al., 2011).

1.1.3. *Alveolar Bone*

The main support of both the teeth and surrounding gingiva is provided by the alveolar bone. The inner part of the alveolar bone, which faces the root cementum, is known as the alveolar bone proper. The regions of the alveolar bone proper where the periodontal ligament fibers (aka Sharpey's fibers) unite the cementum to the bone is known as the bundle bone, which has a very characteristic fascicular arrangement. The periodontal ligament is a highly specialized type of connective tissue that allows for proprioception and physiologic tooth mobility, among other functions. Similar to the supra-alveolar connective tissue attachment (which attaches the tooth to the surrounding gingiva, alveolar crest, and adjacent teeth), the alveolar connective tissue attachment inserts on one side to tooth cementum, and on the other to the bundle bone.

The alveolar bone also consists of two cortical plates (buccal and lingual or palatal), unified at the coronal aspect by the crestal bone, that contains cancellous bone filling the interior space. A thin layer of non-elastic, collagen-rich connective tissue called periosteum covers the outer layer of the buccal cortical bone. The periosteum contains osteoblasts, osteoclasts, precursor cells, blood vessels and nerves to supply the underlying bone (Zuhr and Hürzeler, 2012).

There are indications in the literature that the underlying alveolar bone seems to influence the overlying soft tissue biotype. Interestingly, in an observational study on changes in soft tissue thickness after tooth

extraction, it was observed that the soft tissue thickness was independent of bone thickness. Measurements were made on CBCT images, created with lip retractors in place for visualization of the facial soft tissue. When the study population was divided into a thick (>1mm) or thin (<1mm) buccal bone phenotype, there was no statistically significant difference in buccal soft tissue thickness. The median soft tissue thickness in the thin bone group was 0.7mm versus 0.8mm for the thick bone group. Additionally, no correlation was found between buccal bone and buccal soft tissue thicknesses (Chappuis et al., 2015). Interestingly, the buccal plate thickness and gingival thickness have not consistently shown to be necessarily proportional. In an evaluation of 66 CBCT tooth sites, Frost et al found an association between thin biotype (measured by probe visibility) with a thinner buccal plate thickness by about 0.2mm. However, the comparison was not statistically significant (P=0.06) (Frost et al., 2015).

In a previous study that compared the soft tissue biotype to the underlying alveolar bone, CBCT measurements and clinical measurements were made on cadaver specimens. The biotype was measured by translucency of a periodontal probe in the sulcus. The study teeth, maxillary canine to canine, were extracted and calipers were used to measure the soft and hard tissue thickness, both at 2.0mm apical to the alveolar crest. For facial tissue measurement, there were no statistically significant differences between the CBCT (0.57mm gingiva, 0.94mm bone) and clinical measurements (0.50mm gingiva, 0.83mm bone), validating the radiographic method. The radiographic thickness of buccal soft tissues and bone was only moderately correlated (R=0.429, P=0.000). Visibility of the probe was not predictive of the buccal gingival dimension (Fu et al., 2010). Although commonly used in the clinic, other studies have proven probe visibility is a poor indicator of the gingival thickness dimension (Frost et al., 2015).

1.2. Peri-implant Anatomy

The anatomical structures around a dental implant may appear similar to that around a natural tooth, however there are a few distinct differences. Unlike a tooth, there is an absence of a periodontal ligament joining the implant fixture to the surrounding alveolar bone. Instead, dental implants become osseointegrated with the adjacent bone. As defined by the father of modern dental implantology, PI Brånemark, "a firm,

intimate and lasting connection can be created between the implant and the vital host bone, which remodels in accordance with the masticatory load applied" (Brånemark 1981).

1.2.1. *Peri-implant Biologic Width*

The connection between the osseointegrated implant fixture and the supra-structure can be accomplished in many different ways. The implant surgeon can decide between completely closing the tissue flaps primarily over the implant after placement (two-stage), placing a temporary transgingival healing abutment (one-stage) or immediately placing a provisional restoration. All techniques are similar in that the peri-implant biologic width is established during healing after surgery, as opposed to a developmental process around teeth. The dimensions and material of the transgingival zone can then be finalized to the preferences of the restorative dentist, by means of different abutment systems and retention method of the supra-structure.

The phases of soft tissue healing following one-stage implant placement were explored by Berglundh and colleagues. The researchers placed tissue level implants (with a 2.8mm smooth surface, polished collar) in the mandibles of twenty Labrador dogs. Four days post-surgery, the established blood clot was home to numerous neutrophils, and a dense fibrin network with clusters of leukocytes formed an initial mucosal seal. At one week, the apical portion of the mucosal interface was mostly collagen and fibroblasts. At two weeks, an adherent connective tissue, rich in cells and vascular structures, was covered with an early junctional epithelium. Well organized connective tissue and a larger zone of junctional epithelium was noted at four weeks. The junctional epithelial barrier was mature by six to eight weeks, and maturation of the underlying connective tissue continued through the twelfth week (Berglundh et al., 2007). The practitioner should recognize that there are differences in healing between the canine model and humans, and healing times in the implant clinic are a result of a multitude of different site specific and patient characteristics.

In a previous dog study, Berglundh et al compared the soft tissues adjacent to teeth and integrated implants. Both sites were surrounded by a keratinized oral epithelium which was continuous with a junctional epithelium, terminating about 1-1.5mm coronal to the alveolar crest. Unlike around teeth, the connective tissue around the implants appeared as dense collagen fibers extending from the bone crest to the gingival

margin, aligned parallel to the implant surface (Berglundh et al., 1991). This connective tissue has been compared to scar tissue, composed of a large amount of collagen and small amount of cells and vasculature (Abrahamsson et al., 1996).

In a separate beagle dog study, Berglundh and Lindhe investigated the biologic width around implants where a thin zone of connective tissue was experimentally created at the time of abutment connection. The junctional epithelium was similar between control and experimental sites, 2.1mm versus 2.0mm respectively. The suprabony connective tissue measured 1.8mm at the control sites versus 1.3mm at the test sites. However, at the test sites, where the supra-alveolar mucosa was thinned, the wound healing consistently included bone resorption to form an infrabony defect. (Berglundh and Lindhe, 1996). The authors confirmed a previous hypothesis (Abrahamsson et al., 1996) that a minimum vertical dimension of peri-implant mucosa is necessary, and if this soft tissue dimension was not met initially, bone resorption would ensue to create the necessary biologic width.

Interestingly, similar observations were made in human peri-implant tissue. Using a phase-contrast microscope, Schierano and coworkers examined biopsies of implant abutments and the surrounding 2mm of mucosa. The collagen bundles were observed in three organized orientations; longitudinal fibers which were closest to the abutment, circular fibers which were most abundant, and oblique fibers which were found more proximal seemed to connect the inner fibers to the surrounding submucosa and periosteum (Schierano et al., 2002).

In a human trial, two adjacent implants were placed 2mm supra-crestal and equi-crestal. The implants had a smooth surface coronal collar. The midcrestal tissue thickness was measured before the lingual flap was elevated using a periodontal probe, and sites were divided into thin (<2mm) or thick (>2.5mm). When comparing the implants placed 2mm supra-crestal, sites with thin mucosa demonstrated 1.45±0.55mm of radiographic bone loss versus 0.17±0.19mm of radiographic bone loss in sites with thick tissue at the one-year follow-up. The authors concluded that supracrestal implant placement could not prevent significant crestal bone changes in sites with thin tissue (Linkevicius et al., 2009). In a follow-up study, implants placed in

sites with thin tissue that were simultaneously thickened with a soft tissue allograft behaved similar to sites with initially thick tissue. Sites with initially thin tissue that were not grafted lost a statistically significantly greater amount of bone (1.2±0.08mm) as compared to the thin-grafted and thick groups (0.22±0.06mm). The majority of the crestal bone changes were already apparent at the two-month time point (Puisys and Linkevicius, 2015).

Other factors that can influence biologic width around a dental implant are platform switching (Lazzara and Porter, 2006) and implant abutment material. Abrahamsson demonstrated, in a dog study, that the biologic width around implants from separate manufactures placed according to a one-stage or two-stage protocol resulted in similar composition of peri-implant mucosa (Abrahamsson et al., 1996). However, in a later study with similar design, Abrahamsson et al demonstrated variations in the formation of biologic width around abutments of different material placed on external hex implants in a two-stage protocol. An epithelial and mucosal attachment was observed around titanium and aluminum oxide abutments, whereas no attachment seemed to form around gold and porcelain abutments, leading to soft tissue recession and bone resorption. The mean distance from the abutment-fixture junction to the level of bone-to-implant contact was 0.78±0.17mm in the titanium group, 0.80±0.16mm in the ceramic group, 1.80±0.21mm in the gold group and 1.26±0.31 in the short titanium-porcelain group (Abrahamsson et al., 1998).A later dog study using polarized light and scanning electron microscopy presented evidence of "intense fibroblastic activity" and connective tissue attachment to laser microgrooved abutments, while smooth surface abutments were covered by long junctional epithelium (Nevins et al., 2010). Peri-implant mucosal attachment, regardless of abutment material, is not yet fully understood in humans. However, in human case reports have shown a connective tissue attachment to laser microgrooved abutments (Nevins et al., 2012).

1.2.2. *Peri-implant Keratinized Mucosa*

The width of keratinized tissue is important to implant esthetics, however the necessity of an adequate band of keratinized tissue in order to maintain peri-implant health has been debated. In an earlier implant study, 39 patients with partial and full arch implant restorations were examined at least five years after loading.

Approximately, one quarter of the sites (24%) were completely lacking keratinized mucosa, and 13% of the sites had less than 2mm. Gingival index, probing depth and bleeding on probing were not correlated to either presence or absence of an adequate width of keratinized mucosa (Wennstrom et al., 1994). A systematic review by the same author revealed similar findings regarding keratinized mucosa on peri-implant soft tissue health; health can be maintained irrespective of the keratinized mucosal presence. Additionally, a significant positive correlation between plaque scores and bleeding on probing was found, independent of the type of peri-implant mucosa (Wennstrom and Derks, 2012).

A recent systematic review with meta-analysis identified an association between limited peri-implant keratinized mucosal width (<2mm) with clinical parameters of inflammation. However, likely due to a paucity of long term data rather than a true relationship, there was limited evidence that a lack of keratinized mucosa is predictive of peri-implant attachment loss (Gobbato et al., 2013). A similar association between inadequate keratinized mucosa and peri-implant health has been shown in other publications (Lin et al., 2013, Cairo et al., 2008).

While the debate on the biological need for keratinized mucosa is both interesting and ongoing, the requirement for adequate keratinized mucosa adjacent to the single tooth implant is paramount for an esthetically pleasing outcome. In a retrospective observational study of 41 single tooth implants in 29 patients, the variable with the strongest influence on the clinicians' (five Prosthodontists) overall satisfaction with the restoration was the surrounding soft tissue (Chang et al., 1999). In fact, entire scoring systems have been proposed and validated to objectively rate the esthetic success of peri-implant mucosa (Furhauser et al., 2005, Gehrke et al., 2008, Barwacz et al., 2016).

1.2.3. *Peri-implant Mucosal Thickness*

The bucco-lingual thickness of the peri-implant mucosa has become a more popular topic in dental implant research in recent years. The importance of the peri-implant mucosal thickness (PMT) has proven to be an important characteristic in creating and maintaining ideal esthetics. In a long-term prospective study on immediate implant placement and provisionalization, significantly greater peri-implant facial recession was

observed in patients with thin versus thick biotypes (1.13 ± 0.87mm vs 0.56 ± 0.46mm, $p<0.01$). The biotype was classified before implant placement based on probe visibility (Kan et al., 2011).

Based on the hypothesis that a thicker peri-implant mucosa would mask the unnatural color of implant abutments, Jung developed a rudimentary ***in-vitro*** model using porcine jaws. Block material samples were used to simulate different abutments and connective tissue grafts were placed underneath the mucosa to simulate different peri-implant mucosal thicknesses. The authors found mucosal thickness to be a crucial factor in discoloration caused by the restorative materials (Jung et al., 2007), however the clinical applicability of their model is quite limited. A short time later Jung and collaborators, after evaluating color changes of peri-implant mucosa in thirty humans, did not find differences in relation to mucosal thickness. One possible explanation was the result of the study design; if the peri-implant mucosal thickness measured less than 2mm, the site was augmented with a connective tissue graft, therefore all sites evaluated had a thickness greater than 2mm (Jung et al., 2008).

Bressan and colleagues expanded on a similar hypothesis. Twenty patients receiving a single tooth implant in the anterior maxilla had measurements of color change of the peri-implant mucosa after trying in a gold, titanium or ceramic abutment. Interestingly, the peri-implant mucosal thickness, as measured at the level of the implant collar on a laboratory cast, did not influence the color difference (Bressan et al., 2011).

The relationship between peri-implant mucosal thickness and width has yet to be explored in the literature. One might assume a positive correlation between the two, however this has not been a consistent finding around teeth. Nevertheless, a lack of adequate PMT can lead to a poor subjective assessment of an implant restoration, and this situation has forced many clinicians to innovate techniques to increase the PMT

1.2.4. *Augmenting Peri-implant Mucosa*

Many clinical protocols to enhance peri-implant esthetics by augmenting the peri-implant mucosa have been proposed recently. In an effort to help establish clinical guidelines, Hsu and collaborators stress the importance of the three dimensional location of the implant platform, and recommend autogenous soft

tissue grafting at time of immediate implant placement to compensate for tissue changes during healing (Hsu et al., 2012). Other protocols using autogenous connective tissue at time of placement (Lee et al., 2012, Wiesner et al., 2010, Kan et al., 2009) or to correct a mucogingival defect after restoration with autogenous connective tissue(Zucchelli et al., 2013), or acellular dermal matrix (Mareque-Bueno, 2011) have followed. Not only can augmenting the peri-implant mucosa be beneficial to the esthetic outcome, but also help in maintaining marginal crestal bone levels, as shown by Puisys and Linkevicious (Puisys and Linkevicius, 2015). Systematic reviews have highlighted the heterogeneity of the many protocols and techniques, thus making comparisons on the outcomes challenging (Bassetti et al., 2016, Poskevicius et al., 2015, Thoma et al., 2014a).

1.3 Connective Tissue Grafts

1.3.1. *Subepithelial Connective Tissue Graft*

The subepithelial connective tissue graft (sCTG) has been widely recognized as the gold standard for soft tissue augmentation around teeth, in particular for coverage of recession defects, as confirmed by numerous systematic reviews (Chambrone et al., 2008, Fu et al., 2012, Buti et al., 2013, Chambrone and Tatakis, 2015). It is important to remember that not all palatal connective tissues have similar properties. Variations in patients' anatomy, especially the depth of the palatal vault, location of the greater palatine foramen and thickness of the palatal soft tissue create limitations in the quantity of sCTG available for harvest (Reiser et al., 1996). In a histomorphometric study of palatal connective tissue grafts, a large variation in the fibrous connective tissue thickness (the lamina propria layer) was observed. Unfortunately, thicker palatal tissue correlated with a higher fatty glandular tissue and thinner lamina propria. Lamina propria thickness did not differ significantly throughout different locations in the hard palate, however a deepithelialized connective tissue harvest technique resulted in less fatty glandular tissue than the split-flap technique (Bertl et al., 2015). The maxillary tuberosity is a second location available for connective tissue harvest. Histologic examination of the tuberosity reveals a denser and less vascularized connective tissue as compared to the hard palate. Reports have described a potential hyperplastic healing response leading to an unesthetic, fibrotic overgrowth of tissue when used for root coverage procedures (Dellavia et al., 2014).

Regardless of technique, harvesting an autologous soft tissue graft necessarily entails additional pre-operative preparation, a second surgical site, longer operative time and increased patient morbidity. In a prospective study of 331 consecutive soft tissue procedures in 228 patients, the use of an acellular dermal matrix (ADM) significantly reduced the procedure time, and by replacing an autogenous tissue graft with ADM, the probability of post-op moderate to severe bleeding and swelling was reduced by 70% and 54% respectively (Griffin et al., 2006).

1.3.2. *Acellular Dermal Matrix*

Acellular dermal matrix is essentially donated human dermis, or the connective tissue layer of skin underlying the epithelium. ADM was first used as a treatment for full thickness burns in 1994 (Wainwright, 1995). Commercially available ADM products approved for their use in dentistry include AlloDerm®, Puros® Dermis, Symbios PerioDerm™, Oracell® and SureDerm™ (Silc and Petrungaro, 2013). Each manufacture uses a slightly different proprietary process to remove the epidermis and cells from the donor tissue. AlloDerm (the product used in this study) is sourced from only American Association of Tissue Banks complaint tissue banks and undergoes an extensive panel of serology tests. Processing includes treatment with a buffered salt solution to remove the epidermis, a series of washes with mild non-denaturing detergent solutions to solubilize and eliminate cells, and a patented freeze drying for safe transport and extended shelf life. The final product contains residual antibiotics from processing (BioHorizons Product Data Sheet). This creates a matrix of collagens, elastin, vascular channels and proteins that support revascularization and cell population. The benefits of ADM include an unlimited tissue supply and negating the second palatal surgical site which may reduce surgical time, patient discomfort and post-operative complications. Risks, although rare, include graft site or systemic infection, allergic, hypersensitive or other immune reaction, graft failure and disease transmission.

Several clinical trials comparing ADM and sCTG for the treatment of mucogingival defects around the natural dentition have been performed. Interestingly, a majority observed that ADM produced similar clinical results as compared to sCTG (de Souza et al., 2008, Haghighati et al., 2009, Koudale et al., 2012). Others have

shown similar results in root coverage, but differences in other clinical parameters, such as a greater increase in keratinized tissue in the sCTG group (Paolantonio et al., 2002). A systematic review with meta-analysis confirms these findings, eight randomized clinical trials were included, there were no statistically significant differences between groups for any of the clinical outcomes measured. However, a high level of heterogeneity was found and the total number of sites treated was relatively small in most analyses, so the results should be interpreted with caution (Gapski et al., 2005).

In a randomized controlled clinical trial, Woodyard and colleagues measured the effect of ADM on gingival thickness and root coverage in 24 patients. The control group received a coronally advanced flap alone, while the experimental group received a coronally advanced flap with ADM. An ultrasonic device was used to measure gingival thickness at the sulcus base and mucogingival junction at baseline and six months post-op. The gain in gingival thickness was statistically significant in the experimental group only at both sites; 0.40 ± 0.26mm vs 0.03 ± 0.23mm at the sulcus base and 0.32 ± 0.21mm vs 0.10 ± 0.26mm at the mucogingival junction. The initial gingival thickness at baseline was approximately 0.75 ± 0.20mm at all sites measured. The root coverage was more predictable in the experimental group, 99% vs 67% (Woodyard et al., 2004).

The long-term outcomes of ADM as compared to sCTG are also similar. In a five year randomized clinical trial, a significant loss in root coverage from was observed in both ADM (85.4 ± 22.6% to 54.6 ± 34.9% from 6 to 60 months) and sCTG (69.1 ± 24.3% to 39.8 ± 40.6% from 6 to 60 months), with no statistically significant difference at the 60 month observation (Moslemi et al., 2011).

In a case series of 3 patients as reported by Cummings et al 2005, the histologic differences in healing between ADM and sCTG were evaluated. Hopeless teeth with gingival recession were randomized to ADM, sCTG or coronally advanced flap and extracted en-bloc after six months of healing. It is important to note that all of the patients smoked at least 1 pack per day.

The controls (coronally positioned flap) had parakeratinized epithelium covering the gingiva. There was 0.62 +/- 0.35mm of sulcular epithelium, 1.6 +/- 0.48mm of junctional epithelial attachment and the connective tissue attachment was 0.54 +/- 0.31mm. Dense collagen was present, the fibers were parallel with the root.

The autogenous subepithelial connective tissue grafts had parakeratinized epithelium covering the gingiva. There was 0.57 +/- 0.19mm of sulcular epithelium, 0.97 +/- 0.44mm of junctional epithelial attachment and the connective tissue attachment was 1.04 +/- 0.63mm. Dense collagen was present, the fibers were more disorganized but generally parallel with the root. No differences were found in the grafted area and overlying gingiva, but the graft was distinct from the mucosa. The graft was well incorporated with the periosteal surface.

The ADM had parakeratinized epithelium covering the gingiva. There was 0.47 +/- 0.19mm of sulcular epithelium, 1.17 +/- 0.67mm of junctional epithelial attachment and the connective tissue attachment was 1.13 +/- 0.47mm. Dense collagen was present, the fibers were similar to the overlying connective tissue. Beneath the graft, dense collagen was parallel to the root surface, but perpendicular in one specimen. The authors suspected connective tissue attachment in residual cementum. The ADM appeared as a dense band of collagenous tissue closely approximated to the periosteum. Fibroblasts and small vessels were populating the entire graft. The ADM showed an abundance of elastin after Verhoeff's solution staining. However, the area coronal to the osseous crest and adjacent to the teeth, a layer of dense connective with no elastin fibers were observed. Also, the superficial border of the graft was well incorporated into the overlying mucosa.

No evidence of a major inflammatory reaction was found in any of the groups. As expected, the grafted groups had an increase in tissue thickness (Cummings et al., 2005).

Although it might be logical to infer the concept that ADM, as compared to sCTG, can produce similar clinical results in augmenting peri-implant tissues, there is a paucity of studies comparing the effectiveness of ADM as compared to sCTG around single tooth implant supported restorations.

CHAPTER II. Hypothesis and Specific Aims

It was hypothesized that the use of acellular dermal matrix (ADM) will lead to similar clinical outcomes as compared to the use of autogenous subepithelial connective tissue graft (sCTG) for the augmentation of peri-implant mucosal thickness (PMT) at the time of implant placement.

The purpose was to determine, by way of a non-inferiority trial, the clinical efficacy of ADM in the augmentation of PMT as compared to an autologous sCTG in human adults.

The primary aim of the trial was to assess PMT changes around a single tooth dental implant after following two different clinical protocols (ADM vs sCTG). Secondary aims were to assess changes in keratinized tissue width, healing index, and patient-reported outcomes (i.e. discomfort and satisfaction) following the two different clinical protocols.

CHAPTER III. Material and Methods

3.1. Pre-screening

The clinical component of this trial was conducted in the postdoctoral periodontics clinic at the University of Iowa College of Dentistry and Dental Clinics from January 2015 to June 2016. The University of Iowa Institutional Review Board approval was obtained in November 2014, IRB approval #201407810. This study was registered at clinicaltrials.gov under code NCT02450383.

Patients treatment planned for a single tooth implant, with adjacent natural teeth, at the site of a single-root tooth except for mandibular incisors, were recruited. They were pre-screened by a telephone interview. Individuals who met the pre-screening eligibility criteria were scheduled for a clinical screening.

3.2 Clinical Screening

The clinical screening consisted of an in depth consultation and brief clinical exam. A complete medical and dental history was obtained. Patients were informed of the study purpose, and confirmed they could adhere to the study timeline. Consent was obtained by both written and verbal explanations of the potential risks and benefits of participating, along with other possible treatment options. Ample time was designated for questions and answers, if needed.

Subjects in need of mucosa augmentation at the time of implant placement who met the following inclusion/exclusion criteria were eligible to participate in the study. Inclusion criteria included: between 18 and 80 years of age: at least one single-tooth, non-mandibular incisor edentulous site with adjacent teeth present; thin biotype; planned for an implant supported restoration; patient willing and able to follow instructions related to the study procedures. Subjects must have read, understood and signed an informed consent form.

Exclusion criteria included the following: a reported allergy or hypersensitivity to any of the products to be used in the study; hematologic disorders, such as hemophilia or leukemia; active infectious diseases that could compromise normal healing; uncontrolled systemic disease; liver or kidney dysfunction/failure; current cancer

treatment or within 18 months from completion of radio- or chemotherapy: pregnant or nursing mothers or those who planned on becoming pregnant; smoking within 6 months of study onset; concomitant use of medications for systemic conditions that may affect study outcomes. With regard to uncontrolled systemic disease, subjects with uncontrolled diabetes, defined as Hba1c > 6.5% (According to the most current American Diabetes Association Standards of Care: http://care.diabetesjournals.org/content/suppl/2015/12/21/39.Supplement_1.DC2/2016-Standards-of-Care.pdf) were excluded. Any other non-specified reason that from the point of view of the investigators will make a candidate not a suitable subject for the study (e.g. limited mouth opening).

Provided the patient was able and willing to participate, an intraoral examination was completed. Required site-specific radiographs were ordered as necessary. After verification of the eligibility of the subject, PVS impressions of the arch of interest were obtained in order to fabricate a custom stent for clinical and 3D volumetric measurements. Intraoral photographs were also taken. Subsequent study visits were planned and preoperative instructions were given to subjects (Figures 1 and 2).

If, for any reason, the patient did not qualify for the study, comprehensive dental care at the dental college, but outside the study, was offered.

3.3 Calibration

The masked examiner (K.T.) was familiarized with the technique of measuring gingival thickness using a custom stent and endodontic instrument. Gingival thickness measurements, obtained in identical fashion to the measurements made on the study subjects, were made at three separate locations using three separate custom made stents on one volunteer. The measurements were repeated 14 days later, and the results analyzed for intraclass correlation.

3.4 Randomization

Before the baseline surgical intervention visit, each subject was randomly assigned with the assistance of computer software to one of the following groups: Control Group: Implant placement with simultaneous

autogenous sCTG harvested from the palate, or, when possible, the maxillary tuberosity. Experimental Group: Implant placement with simultaneous ADM (AlloDerm, BioHorizons, Inc.) The allocation was withheld from the surgeon (C.H.) until shortly before the surgical visit. The clinical examiner (K.T.) was masked to the treatment group for the duration of the study.

3.5. Baseline Measurements

After a sufficient amount of Lidocaine 2% with Epinephrine 1:100,100 local anesthetic was administered, the buccal PMT was measured and recorded. Gingival and plaque indices were recorded, as well (Loe, 1967, Silness and Loe, 1964) (Figure 3). A periodontal probe and endodontic spreader were gently inserted to the gingiva and bone perpendicular to the facial aspect of the tooth to measure the PMT at points approximately 1, 3 and 5 mm apical to the estimated free gingival margin (based on the gingival zenith of the adjacent teeth). The difference between the measurement from the stent to the gingiva (using the probe) and from the stent to the bone (using an endodontic spreader) resulted in the PMT. A custom stent was used to assure the mesial-distal and apico-coronal locations are consistent between appointments (Figures 4 and 5).

3.6. Surgical Intervention

After the baseline measurements were recorded and adequate local anesthesia was confirmed, a crestal incision slightly offset to the palate was made, with attempt to ensure at least 2mm of buccal keratinized tissue was maintained on the facial. The implant drill sequencing and placement followed the implant manufacturer's instructions (Tapered Internal Plus®, BioHorizons Inc., Birmingham, AL), and a cover screw was placed.

A combination of full thickness and partial thickness flap design was used to create a recipient bed for the graft. For the control group, a connective tissue graft was harvested from the palate, or maxillary tuberosity if possible. For the test group, the ADM was prepared according to the manufacturer's recommendations and trimmed to appropriate size. Care was taken to assure the size of the ADM resembled a reasonably attainable connective tissue graft. The grafts were positioned over the coronal and buccal aspect of the

alveolar ridge and secured to the recipient sites with absorbable chromic-gut mattress sutures anchored in the buccal periosteum and palatal flap and then passively covered with the flap. A periosteal releasing incision helped insure tension free primary closure. The flaps were sutured with multiple simple interrupted and double sling (Wachtel et al., 2006) sutures. Digital pressure was applied to thin the clot between graft and recipient bed (Figures 6 and 7).

3.7. Post-operative Care

Subjects received detailed written and verbal post-operative instructions. Subjects were instructed to avoid mechanical disturbance of the surgical site for the first week. Oral hygiene instructions included 0.12% chlorhexidine mouth rinses after 48 hours and no direct brushing of the surgical site for one week. All subjects were prescribed oral antibiotics. Amoxicillin 500 mg every 8 hours for 7 days was the medication of choice. If an allergy to this antibiotic is reported Clindamycin 300 mg every 8 hours for 7 days was prescribed. An anti-inflammatory and pain reliever drug (Ibuprofen 600 mg every 4-6 hours for 3 to 5 days), and narcotic pain reliever (Hydrocodone/APAP 5/325 mg every 6-8 hours as needed for pain, up to 4 days) was prescribed to all subjects, unless contraindicated for individual medical reasons.

3.8. Follow-up Visits and Assessments

Subjects returned to the clinic at 2, 4, 8 and 16 weeks. Sutures were removed at 2 weeks postoperatively. At every follow-up visit, intraoral photographs were taken, modified wound healing index (MWHI) assessments were recorded and oral hygiene instructions were reinforced (Figure 8).

At the 16-week visit, all clinical measurements were repeated. After achieving adequate local anesthesia, the buccal PMT was again measured with a periodontal probe and endodontic spreader. At this time, the second stage implant uncovering surgery was completed.

At all postoperative visits subjects were asked to score their perceived pain by using a visual analog scale (VAS) of 100 points. Using the same scale, an overall satisfaction score was obtained at the 16 week visit only (Figures 9 and 10).

3.9. Power Analysis

Sample size calculations were focused on providing adequate power for the primary outcome, change in PMT expressed as the proportional change in mm from baseline to 16 weeks for each of the two treatment groups (experimental and control).

In a previous case series, Puisys et al measured mean peri-implant tissue thickness of thin coronal tissue that was augmented with ADM over implants that were allowed to heal with a cover screw (Puisys et al., 2014). Cases with initially thin soft tissue before augmentation had an average crestal thickness of 1.54 ± 0.51mm and after augmentation the thickness increased to 3.75 ± 0.54mm.

These values were employed, assuming a normal distribution to perform a sample size calculation using an open-source software package (Faul et al., 2007). If the true mean difference between the experiment and control sites at 16 weeks is 1.0mm and the subsequent effect size is 1.4, ten experimental subjects and ten control subjects were needed to be able to reject the null hypothesis that the population means of the experimental and control groups are equal with probability (power) 80%. The Type I error probability associated with this test of the null hypothesis is 0.05.

3.10. Statistical Analysis

Statistical analyses were performed with for the change in the primary outcome of peri-implant mucosal thickness; changes in keratinized mucosal width and in the other clinical and patient related parameters were also analyzed. A p-value of < 0.05 was considered statistically significant. A Pearson correlation test was used to determine intra-examiner reliability. Student's T-Tests were used to compare differences in age and BMI between experimental and control groups. Mann Whitney U tests were used to test for differences in all other clinical and patient related outcomes. The Mann Whitney U test was chosen because normal distribution was not assumed, the sample sizes in the control and experimental groups were small, and the standard deviation in the PMT and KMW measurements were relatively large.

CHAPTER IV. Results

4.1. Examiner Calibration

A total of 18 measurements were made (9 soft tissue, 9 bone) in each session separated by 14 days. The Pearson's r (correlation coefficient) was 0.96, demonstrating a very strong intra-examiner reliability (Figure 11).

4.2. Subject Population

Thirty patients were appointed for clinical screening. Five patients decided not to participate in the study after a thorough explanation of the surgical procedure and follow-up schedule. One patient did not qualify due to systemic health concerns, one patient lacked adequate spacing between adjacent tooth roots for a dental implant, and three patients were not accepted as they required ridge augmentation prior to implant placement. Twenty patients were ultimately accepted (10 control, 10 experimental); of which fifteen (6 control, 9 experimental) have completed the study to date and were included in the data analysis hereby presented.

4.3. Subject Characteristics

The mean age of the subjects was 56.3 ± 11.3 years (control: 49.3 ± 9.0, experimental: 60.9 ± 10.3). In total, 9 males and 6 females completed the study (control: 3 males, 3 females, experimental: 6 males, 3 females). The mean Body Mass Index (BMI) was 30.9 ± 4.7 (control: 28.6 ± 2.7, experimental: 32.4 ± 5.1) (Table 1). The difference in age or BMI was not statistically significant (Student T-test; age $p=0.057$ and BMI $p=0.1468$).

4.4. Baseline Data

The sites treated in the study included 6 central incisors, 2 lateral incisors, 1 canine and 6 second premolars (control: 2 central incisors, 1 lateral incisor, 1 canine and 2 second premolars; experimental: 4 central incisors, 1 lateral incisor, 4 second premolars). The average Plaque Index (PI) score for the control group was 0.8 (mesial: 0.5, distal: 1.2) and for the experimental group was 0.9 (mesial: 0.9, distal: 0.9). The average Gingival

Index (GI) score for the control group was 0.7 (mesial: 0.8, distal: 0.5) and for the experimental group was 0.4 (mesial: 0.4, distal: 0.3). The differences between groups for PI and GI were not significant (Mann-Whitney test; PI mesial p=0.399, PI distal p=0.569, GI mesial p=0.146 and GI distal p=0.533).

The average baseline keratinized mucosal width (KMW) was 6.00 ± 0.82mm in the control group and 4.89 ± 1.37mm in the experimental group. This difference was not statistically significant (Mann-Whitney U=15, p=.174). The average baseline peri-implant mucosal thickness (PMT) in the control group was 2.33 ± 0.99mm at 1mm apical to the CEJ, 2.50 ± 0.96mm at 3mm apical to the CEJ and 1.75 ± 0.85mm at 5mm apical to the CEJ. The average baseline peri-implant mucosal thickness (PMT) in the experimental group was 2.89 ± 1.39mm at 1mm apical to the CEJ, 2.33 ± 1.00mm at 3mm apical to the CEJ and 1.72 ± 0.67mm at 5mm apical to the CEJ. (Table 2). In summary, the baseline PMT measurements were not significantly different between groups.

4.5. Wound Healing

In the control group, 3 minor post-operative complications were noted (2 exposures of the graft due to wound dehiscence and 1 incidence of palatal bone exposure at the donor site). In the test group, 7 minor post-operative complications were noted (7 exposures of the graft due to wound dehiscence of varying size). All complications were resolved by the 8-week follow-up. The average Modified Wound Healing Scale (MWHS) score at the 2-week follow-up was 1.67 in the control group and 2.00 in the experimental group. The average MWHS score at the 4-week follow-up was 1.00 in the control group and 1.56 in the experimental group, at the 8-week follow-up was 1.00 in the control group and 1.11 in the experimental group. At the 16-week follow-up the MWHS score was 1.00 for both the control and experimental groups. The MWHS was significantly lower at the 4-week follow-up for the control group compared to the experimental group (Mann-Whitney U=12 p= .031). The healing scores at all other time points were not statistically different between the control and experimental groups (Table 3).

4.6. Patient Reported Outcomes

A Visual Analog Score (VAS) was used for patients to rate their discomfort at the follow-up visits. The average discomfort score at the 2-week follow-up was 27.0 ± 31.7 in the control group and 9.7 ± 8.1 in the experimental group. The average discomfort score at the 4-week follow-up was 15.0 ± 20.5 in the control group and 4.8 ± 4.3 in the experimental group, at the 8-week follow-up was 14.7 ± 18.9 in the control group and 4.8 ± 8.3 in the experimental group, and at the 16-week follow-up was 10.8 ± 19.2 in the control group and 7.3 ± 9.9 in the experimental. At the 16-week follow-up patients were also asked to rate their overall satisfaction in the study. The control group reported an average satisfaction of 99.0 ± 2.0 and the experimental group reported an average satisfaction of 94.1 ± 7.2. There were no statistically significant differences in the patient reported outcomes. However, assuming satisfaction scores as ordinal data, the difference between the control and experimental patient reported satisfaction becomes significant (Mann-Whitney U=9.5, p= .035) in favor of the control group (Table 4).

4.7. 16-week Follow-up Data

Using the same custom surgical stent employed at baseline, the clinical measurements of KMW and PMT were repeated at 16 weeks. The average 16-week keratinized mucosal width was 5.08 ± 0.66mm in the control group and 4.61 ± 0.92 in the experimental group (Table 5).

The average 16-week peri-implant mucosal thickness (PMT) in the control group was 4.10 ± 0.93mm at 1mm apical to the CEJ, 4.58 ± 1.53mm at 3mm apical to the CEJ and 2.58 ± 0.38mm at 5mm apical to the CEJ. The average 16-week PMT in the experimental group was 3.00 ± 1.27mm at 1mm apical to the CEJ, 3.17 ± 1.35mm at 3mm apical to the CEJ and 3.28 ± 0.91mm at 5mm apical to the CEJ (Table 6).

The gain (or loss) in KMW and PMT were calculated by taking the difference between the 16-week and initial measurements. The control group, on average, lost 0.92 ± 1.02mm of KMW and the experimental

group lost 0.28 ± 1.25mm of KMW. The loss of KMW was not statistically significantly different between groups (Mann-Whitney U=19.5 p=0.366) (Figure 12).

The gain in PMT in the control group was 1.70 ± 1.92mm at 1mm apical to the CEJ, 2.08 ± 0.80mm at 3mm apical to the CEJ and 0.83 ± 0.75mm at 5mm apical to the CEJ. The gain in PMT in the experimental group was 0.11 ± 1.65mm at 1mm apical to the CEJ, 0.83 ± 1.37mm at 3mm apical to the CEJ and 1.56 ± 1.18mm at 5mm apical to the CEJ. The gain in PMT 3mm from the CEJ was statistically significantly better in the control group (Mann-Whitney U= 10.0 p= .043), while the PMT gains at 1mm and 5mm from the CEJ were not statistically different (Figure 13).

A Spearman's correlation was used to determine the relationship between the initial width of KMW and changes in PMT. The initial KMW width did not seem to have an effect on the change in PMT. Interestingly, in the experimental group, a statistically significant negative correlation existed between the initial KMW and the change in KMW (r= -0.78, p=0.013). This relationship was not found in the control group.

The effect of the baseline BMI on the change in PMT was also analyzed for correlation. In general, there were no statistically significant correlations between BMI and PMT with the exception of the change at 5mm apical to the CEJ in the control group only (r=0-.971, p=0.001). A similar analysis was completed to determine the effect of age on PMT changes. Similarly, there were no statistically significant correlations between age and PMT with the exception of the change at 5mm apical to the CEJ in the experimental group only (r=0.841, p=0.004).

CHAPTER V. Discussion

The primary aim of this study was to assess peri-implant mucosal thickness (PMT) changes around a single tooth dental implant after following two different clinical protocols, the use of autologous sub-epithelial connective tissue (sCTG) or the use of an acellular dermal matrix substitute (ADM). Secondary aims were to assess changes in keratinized mucosal width (KMW), healing index, and patient-reported outcomes.

The mean gain in PMT was approximately 1.5mm in the control group and 0.8mm in the experimental group. When measured at 1, 3 and 5mm apical from the CEJ, only at the 3mm site was the difference between the groups statistically significant (control: 2.08 ± 0.80mm, test: 0.83 ± 1.37mm, Mann Whitney U = 10.00, p= 0.043) Changes in KMW, healing index and patient reported outcomes were generally similar between the two groups.

The mean baseline PMT was approximately 2.25mm overall, 2.6mm 1mm apical to the CEJ, 2.4mm 3mm apical to the CEJ and 1.74mm 5mm apical to the CEJ. The greatest measurement of baseline PMT was 5.0mm (measured 1mm apical to the CEJ) and the smallest measurement was 0.5mm (also at 1mm apical to the CEJ). The average PMT is in accordance with, although slightly greater than, other reports in the literature. Goaslind reported a mean thickness at the depth of the gingival sulcus of 1.56 ± 0 .39mm (ranging from 0.53mm to 2.62mm) and a mean thickness of 1.25 ± 0.42mm at the midway point between the depth of the sulcus and the mucogingival junction (ranging from 0.43mm to 2.29mm) (Goaslind et al., 1977). In a separate study the facial gingiva, measured at 1 to 2mm apical to the gingival margin, ranged from 0.70±0.15mm at maxillary canines, 081±0.28mm at maxillary second premolars, 0.84±0.21mm at maxillary first premolars, 0.86±0.33mm at maxillary lateral incisors, and 1.00±0.30mm at maxillary central incisors (Muller et al., 2000b). The differences in measurement techniques are bound to reproduce slightly different means in facial tissue thickness, considering differences are generally about 0.5mm or less, which is difficult to measure in the dental clinic. The measurements reported above have smaller standard deviations compared to the results of this study, likely due to the use of potentially more accurate measurement instruments

(Goaslind: transformer probe, Muller: Ultrasonic device) and larger sample sizes (Goaslind: 10 subjects, 10 sites per subject, Muller: 40 patients, at least 4 buccal sites per tooth).

All study sites were healed ridges bound by natural teeth and most sites were preserved by socket grafting at time of extraction. A thicker crestal tissue can be explained by the volume compensation of the soft tissue in thin bone phenotypes as explained in a recent post-extraction analysis. In non-grafted sites with an initially thin bone phenotype, the soft tissue thickness increased an average of 4.8mm (Chappuis et al., 2015). Although a smaller soft tissue compensation than described by Chappuis is expected in ridge preservation sites, this can partially explain the greater initial PMT at the more coronal levels. Extremes in soft tissue compensation observed within months after tooth extraction is one reason the proposed implant site needed to be a healed alveolar ridge in order to meet the inclusion criteria, as a greater risk in increased variation of the soft tissue dimension after tooth extraction and after immediate implant placement is expected. Despite the protocols set forth in the study design to reduce the variation in treatment sites between subjects (i.e. single-tooth, non-mandibular incisor edentulous site with adjacent teeth present, with sufficient healing of the ridge or ridge preservation bone graft for implant placement, the variation in buccal) bone thickness has likely contributed to the variation in soft tissue thickness. This is especially true at the 1mm apical to the CEJ measurement site, as wider variation in buccal bone thickness at the crest of the ridge is expected both after healing from tooth extraction and ridge preservation, and after implant placement. At the time of uncovering, flap reflection to evaluate the buccal bone crest was not performed, the status of buccal bone was only confirmed by sounding with a periodontal probe. This potential variation in buccal bone morphology likely contributes to the large variation in changes of PMT 1mm apical to the CEJ.

While abundant literature is available on the independent or comparative use of sCTG and ADM, particularly around natural teeth, studies comparing techniques to augment PMT are lacking. In a systematic review of assessing different techniques to augment the soft tissue width and thickness around implants and in partially edentulous ridges, the sCTG was recommended to be the "treatment of choice". The range of increase in soft tissue thickness was 0.35 – 3.2mm, with a volume increase regardless of the technique or material.

Despite these findings, the authors note soft tissue substitutes "lack clinical data and can currently not be recommended". The authors also pointed out the paucity of evidence on the shrinkage of the gain in volume (Thoma et al., 2014a).

In another systematic review comparing protocols for soft tissue thickness augmentation, either at implant placement or around existing dental implant, a more predictable gain was observed when grafting was completed around existing implants (volume gain of 0.8 to 1.4mm) as compared to grafting at time of implant placement, in a simultaneous approach (loss of 0.25 – gain of 1.43). Interestingly, both techniques led to a gain in KMW of approximately 2.5mm. The review only included autogenous grafting techniques. The authors concluded that due to heterogeneity between studies, it is difficult to recommend a universal technique for every case (Poskevicius et al., 2015). The gains in PMT thickness obtained in this study (~1.5mm in the control group and 0.8mm in the experimental group) fall into the range of the few reports available for comparison in the literature.

The greatest mean gain in this study, 2.08 ± 0.80mm was observed in the sCTG group, 3mm apical to the CEJ. The least gain was observed in the ADM group, 0.11 ± 1.65mm, 1mm apical to the CEJ. This is likely correlated to the high incidence of post-operative complications in the experimental group, all of which were tissue dehiscences of varying dimensions. The exposed allograft portion was often, but not always, partially removed under local anesthesia to prevent infection of the whole graft. The decision to trim exposed autograft was never made in the 2 instances in which this complication was observed, as the sCTG was incorporating adequately in absence of signs of infection by the first follow-up (at 2 weeks).

The high rate of exposures can be explained by three variables. First, and likely most influential, was the flap design. Without release of the papillae, advancing the buccal aspect of the crestal incision back to the static palatal flap was difficult. While the influence of flap tension on outcomes of periodontal surgery is difficult to quantify due to other confounding factors, in general a tension free primary closure is recommended, especially in periodontal plastic surgery (Pini Prato et al., 2000). A periosteal release was required to obtain passive primary closure, severing principal vasculature to the coronal aspect of the flap (Mormann and

Ciancio, 1977). Second is the relative inexperience of the surgeon (C.H.), a Periodontics resident. Nevertheless, every attempt was made to assure adequate flap release and passive primary closure. Third is the location of the graft. The graft was mostly covered by the buccal flap, as the incision was offset slightly towards the palate, and the graft was positioned to cover the crest and buccal ridge as a "J" shape. The vascularity to the buccal flap was compromised by the periosteal releasing incision. Also, the intaglio portion of the graft at the coronal aspect of the ridge was placed on denuded bone and a cover screw, limiting blood supply form both sides of the graft as no periodontal ligament exists adjacent to the dental implant. In a classic animal study, Caffesse and collaborators histologically demonstrated a free autogenous gingival graft heals in similar patterns over periosteum and bone. The main differences were limited to the first 7 days, where better graft to recipient site incorporation was noted early on, and bone resorption was apparent when the graft was placed on denuded bone (Caffesse et al., 1979). In a single case study, placement of ADM onto a large area of denuded bone created a post-operative course that resembled a sloughing graft and a denudation procedure. Despite the author speculating the ADM merely acted as a bandage (supported by histology, there was no evidence of the graft in the post-op biopsy), the patient ultimately healed well and was satisfied with the outcome (Harris, 2001). Harris published a very similar study a few years later, although the ADM was placed on periosteum. The goal of the surgery, obtaining a gain in keratinized gingiva, was not met. However, histologic evidence of graft incorporation/ repopulation was observed (Harris, 2004). Interestingly, ADM has been shown, on limited case reports, to increase the width of keratinized mucosa. In a case series of 10 patients, placing the ADM beneath a partial thickness flap adjacent to dental implants, the width of keratinized mucosa increased from 0.8 ± 0.6mm to 2.2 ± 0.6mm at six months (Park, 2006). Placing ADM over denuded bone and/or other avascular surfaces seems to be a major risk factor for exposure and subsequent infection. In a case series using ADM as a barrier between the buccal flap and bone particular allograft to cover implant dehiscences, ADM exposure was reported in 3 of the 5 patients (Park and Wang, 2006).

The apparent decrease in the gain of PMT at 5mm apical to the CEJ in the control group is likely due to the more irregular morphology of the harvested sCTG and its displacement respective to baseline upon suturing.

The mesial-distal dimension of the donor tissue became the apico-coronal dimension over the implant site, and the relative lack of experience of the surgeon likely resulted in sCTGs that did not consistently extend beyond 5mm past the proposed CEJ. In addition, 3 sCTGs were harvested from the tuberosity, where availability of connective tissue harvesting is highly patient specific. Regardless, the most demanding location for thick PMT in order to preserve marginal bone levels and esthetics is at the CEJ and transmucosal region, which was repeatedly accomplished.

The loss of KMW is attributable to the flap advancement over the connective tissue graft. The commercially available ADM used in this study had a reported thickness of 0.9 – 1.6mm and the size of the control and experimental grafts were kept to similar dimensions. Despite the relatively thin dimension of the graft, considerable flap advancement was necessary for primary closure. An interesting finding was the greater loss of KMW in the control group (although not statistically significant, the control group, on average, lost 0.92 ± 1.02mm of KMW and the experimental group lost 0.28 ± 1.25mm KMW). In a classic study on changes in epithelium after gingival grafting, (Karring et al., 1975) demonstrated the ability of the grafted connective tissue to influence the overlying epithelium (when placed into a mucosal pouch and later de-epithelialized after healing). Due to the complete lack of cells in the ADM, the neighboring cells that eventually populate the graft are responsible for the epithelial signaling.

KMW has also been studied to determine its correlation with biotype and outcomes of gingival grafting. While the facial mucosal thickness has been repeatability directly correlated with gingival grafting outcomes (Hwang and Wang, 2006, Baldi et al., 1999), it has not been consistently associated with KMW (Goaslind et al., 1977, Muller et al., 2000a) or bone biotype. In this study, there was no appreciable effect of the baseline KMW on the changes in PMT. The baseline KMW was only statistically significantly correlated between the initial KMW and the change in KMW ($r = -.78$, $p = .013$) in the experimental group. Because no other correlations were found using KMW as the dependent variable, it is likely this finding is a result of the difficult obtaining the true KMW when measuring from the FGM to the ridge crest at a partially edentulous site. Regardless, the absolute necessity of keratinized mucosa around dental implants remains somewhat

controversial, although it seems to be protective against local inflammation (Gobbato et al., 2013) and necessary for esthetics (Furhauser et al., 2005).

While obesity has been identified for increased risk for systemic disease and adverse outcomes of major surgery, its role in adverse outcomes in oral surgery remains debatable (Waisath et al., 2009). In this study, there were no statistically significant correlations between BMI and PMT with the exception of the change at 5mm apical to the CEJ in the control group only ($r=-.971$, $p=.001$). Again this exception to the normal findings may be explained by the low sample size and large variance in changes in PMT. The analysis of the effect of age on PMT changes rendered similar results. No statistically significant correlations between age and PMT changes were noted, with the exception of the change at 5mm apical to the CEJ in the experimental group only ($r=.841$, $p=.004$). Additionally, the only statistically significant differences in healing was at the 4-week follow-up (lower MWHS for the control group compared to the experimental group (Mann Whitney $U = 12.00$, $p= .031$). Perhaps due to the high incidence of wound dehiscence and lack of cellularity of the ADM, the sCTG seemed to heal faster, especially when exposed. This may also explain the statistically significant difference in the patient's overall satisfaction in the study. The additional procedure required to manage the exposed ADM in those patients in the experimental group may have detrimentally affected not only the healing score at 4 weeks, but the overall experience in the study. The control group reported an average satisfaction of 99.0 ± 2.0 (median 100) and the experimental group reported an average satisfaction of 94.1 ± 7.2 (median 98), the difference between groups was statistically significant (Mann-Whitney $U=9.5$, $p= .035$) in favor of the control group.

One interpretation on this data is that, despite the high incidence of minor post-operative complications, the overall procedure is relatively safe, as it was tolerated by both older and obese subjects equally as well as younger healthy subjects. However, the low sample size should again be stressed when drawing conclusions.

Future directions in this field of dental implant research should be directed at stability of graft volume over time, outcomes on implant stability and the effect on implant esthetics. The long term stability of of ADM and sCTG was demonstrated in a 5-year split mouth trial. The correction of gingival recession around teeth

was shown to relapse significantly from baseline, about 1mm in each treatment group (with no significant differences between groups) and was associated with horizontal, traumatic tooth brushing techniques (Moslemi et al., 2011). A systematic review of soft tissue augmentation at second-stage surgery reported approximately 30-40% shrinkage of the KMW after 3 months and up to 50% after 6 months. The reduction in the soft tissue volume was only reported in one study and amounted to about 0.25mm when a connective tissue graft was used and 0.5mm in a roll-flap technique. Similar results were published when augmenting alveolar ridge defects, about 40% shrinkage with ADM (Batista et al., 2001) and 50% with sCTG (Akcali et al., 2015) at 6 months. Reports have shown the implants placed adjacent to thick tissues, or initially then and augmented tissues have less crestal bone loss than thin tissues that are not grafted. Thicker PMT is also associated with better esthetics (Thoma et al., 2014b) and less risk for tissue recession, especially in immediate implants (Evans and Chen, 2008, Kan et al., 2011). Unfortunately, these clinical measures were not part of this study protocol.

CHAPTER VI. Conclusions

The purpose of this study was to determine, by way of a non-inferiority trial, the clinical efficacy of ADM in the augmentation of PMT as compared to an autologous sCTG in human adults. A large variance in primary outcome measures and small sample size were limiting factors in the data analysis. The clinical impression of the difference between the two treatment groups was negligible. It appears ADM or sCTG can be feasibly used to increase the PMT at time of implant placement.

APPENDIX A – Figures

Figure A1. Study Timeline

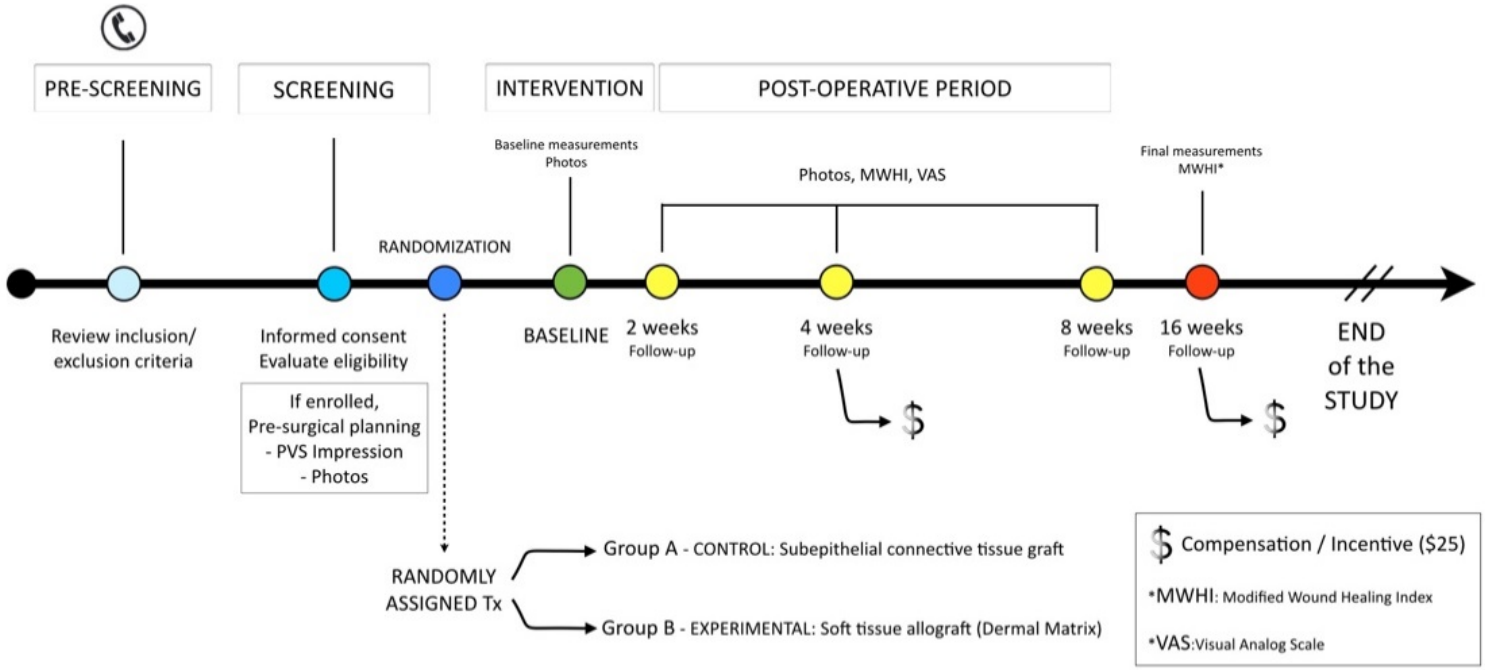


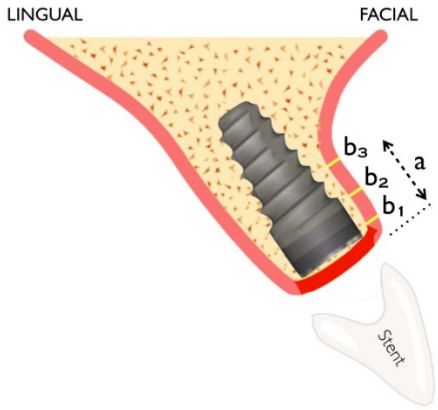
Figure A2. Events Schedule

Visit	1	2	5	6	7	8
Visit Description	Screening	Mucogingival surgery (MG Sx)	Follow-up #1	Follow-up #2	Follow-up #3	Follow-up #4
Visit Window	-	-	MG Sx + 2 weeks (±2 days)	MG Sx + 4 weeks (±4 days)	MG Sx + 8 weeks (±4 days)	MG Sx + 16 weeks (±7 days)
Informed Consent	X					
Update Medical/Dental History	X	X	X	X	X	X
Inclusion/Exclusion Criteria	X					
Intraoral Examination	X	X	X	X	X	X
PVS Impression	X					
Clinical Photographs	X	X	X	X	X	X
Clinical Measurements KMW/KMT		X				X
Modified Wound Healing Index			X	X	X	X
Adverse Events & Adverse Device Effects		X	X	X	X	X
Length of Visit (Estimated)	1 to 1.5 hours	2 to 3 hours	15-30 minutes	15-30 minutes	15-30 minutes	30 minutes

Figure A3. Plaque Index and Gingival Index

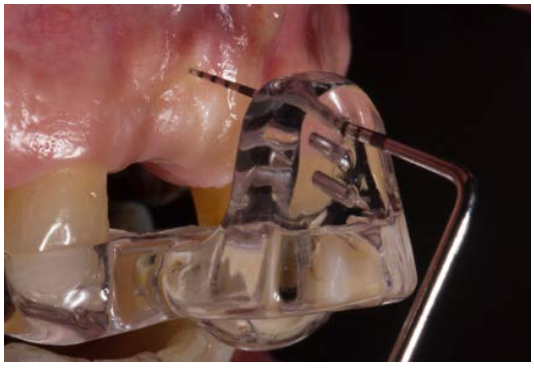
PLAQUE INDEX (Loe 1964)								**GINGIVAL INDEX** (Loe 1967)							
0 No plaque		**1** Film of plaque detected by probe		**2** Plaque detected by naked eye		**3** Abundance of soft matter		**0** Normal gingiva		**1** Mild inflammation		**2** Moderate inflammation		**3** Severe inflammation	
B	**L**	**B**	**L**	**B**	**L**	**B**	**L**	**B**	**L**	**B**	**L**	**B**	**L**	**B**	**L**

Figure A4. Clinical Measurements Diagram

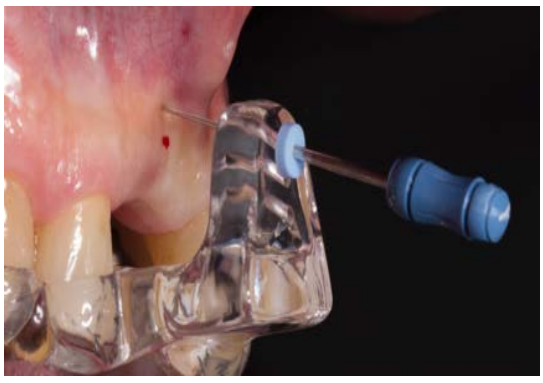


a - Width of facial KM (using a stent)
b_1 - Facial mucosa thickness 1 mm apical from the mucosal margin
b_2 - Facial mucosa thickness 3 mm apical from the mucosal margin
b_3 - Facial mucosa thickness 5 mm apical from the mucosal margin

Figure A5. Clinical Measurements Photos



Soft tissue measurement, 5mm apical to CEJ



Hard tissue measurement, 5mm apical to CEJ

Figure A6. Example Surgery - Control

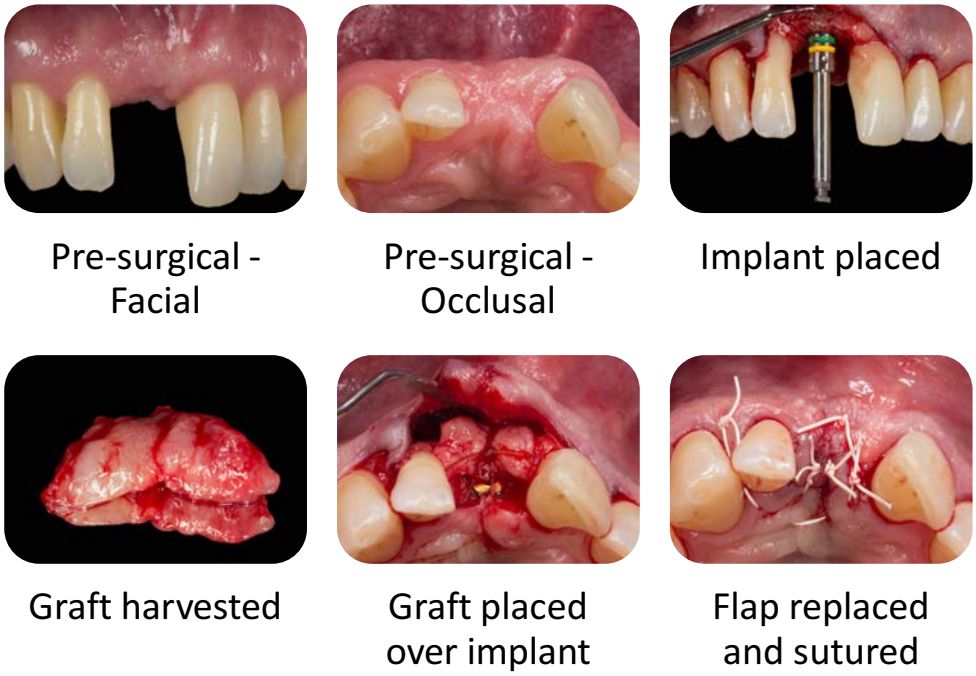


Figure A7. Example Surgery - Experimental

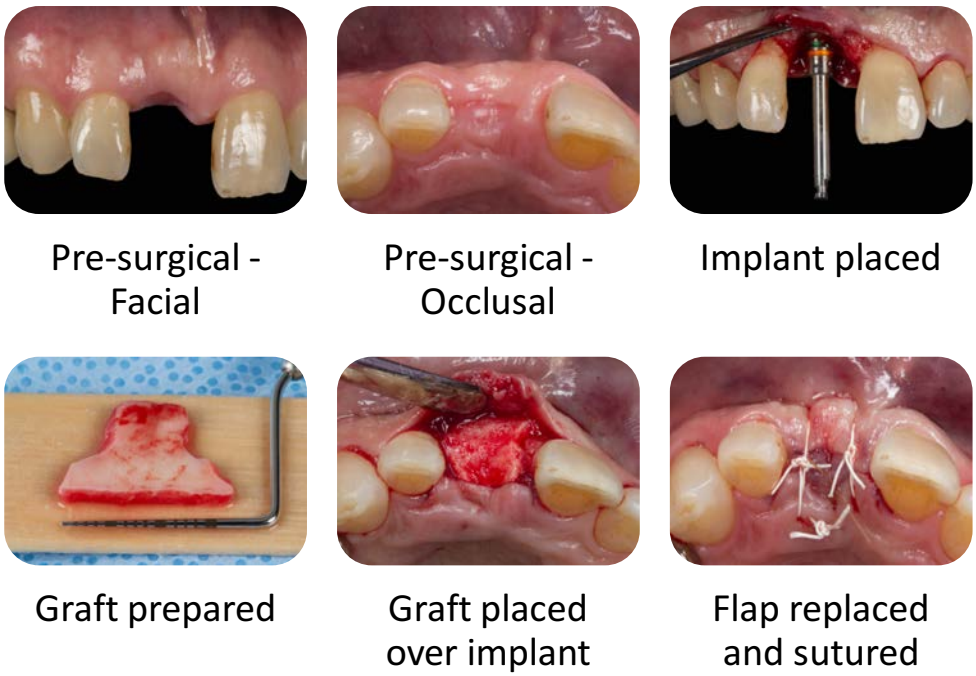


Figure A8. Modified Wound Healing Scale

Modified Wound Healing Scale (Mod-CWHS)	
1	Uneventful wound healing with no gingival edema, erythema, suppuration, discomfort or graft exposure
2	Uneventful wound healing with slight gingival edema, erythema, or discomfort but no suppuration
3	Poor wound healing with significant gingival edema, erythema, discomfort, loss of graft or any suppuration

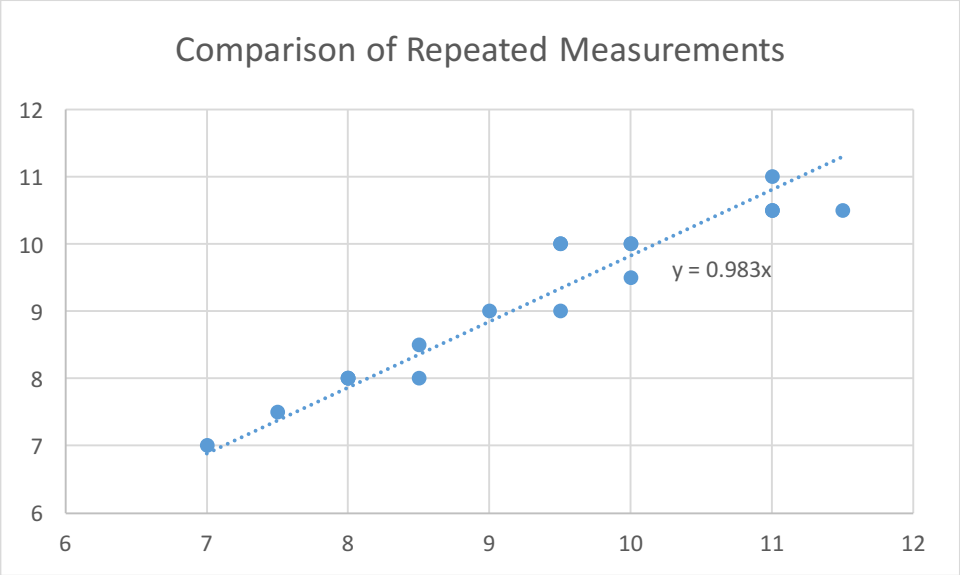
Figure A9. VAS Scale - Patient Discomfort

DISCOMFORT
Less ---------- More

Figure A10. VAS Scale - Patient Satisfaction

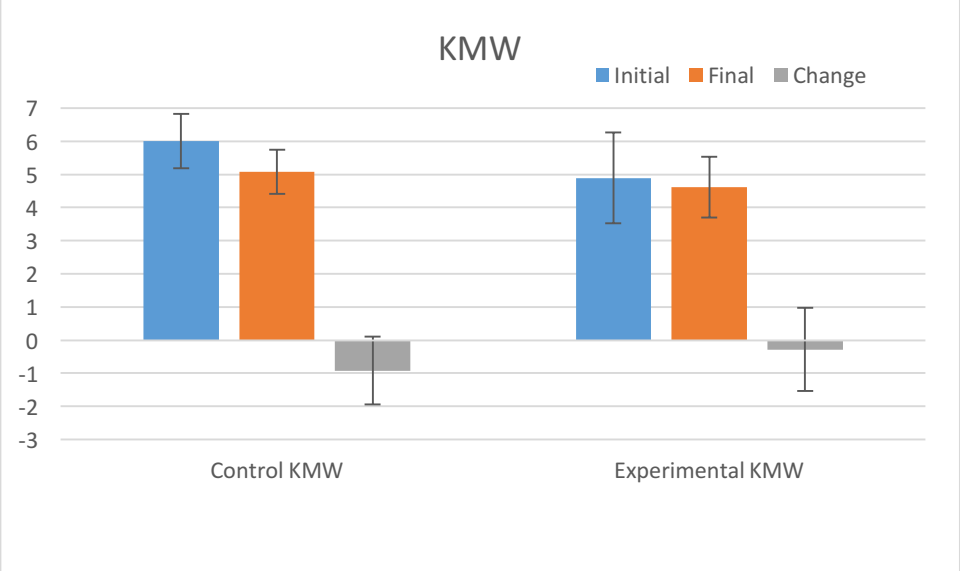
How satisfied are you after participating in the study?
Less ---------- More

Figure A11. Scatter Plot - Calibration Measurements



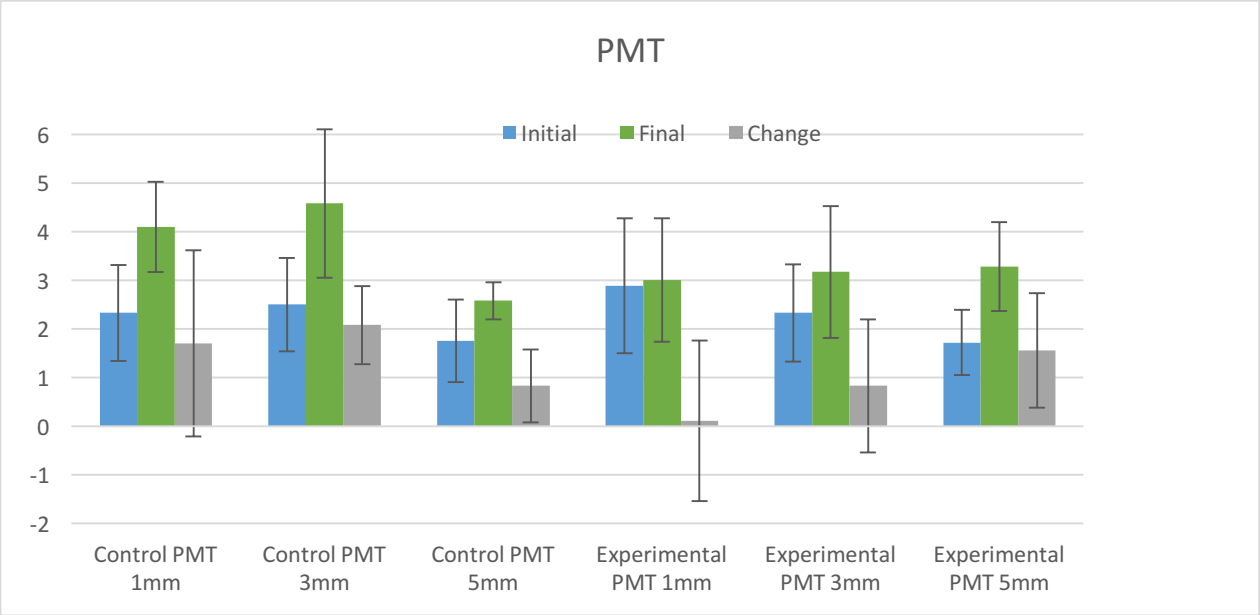
Measurements repeated after 14 days

Figure A12. Changes in KMW



(Error bars are standard deviation)

Figure A13. Changes in PMT



(Error bars are standard deviation)

APPENDIX B – Tables

Table B1. Subject Characteristics

Subject Characteristics	Age	Gender	BMI
Overall	56.3 ± 11.3	F-6 M-9	30.9 ± 4.7
Control	49.3 ± 9.0	F-3 M-3	28.6 ± 2.7
Experimental	60.9 ± 10.3	F-3 M-6	32.4 ± 5.1

Table B2. Baseline Data

Baseline Data	PI	GI	KMW	PMT 1mm	PMT 3mm	PMT 5mm
Control	0.8	0.7	6.00 ± 0.82mm	2.33 ± 0.99mm	2.50 ± 0.96mm	1.75 ± 0.85mm
Experimental	0.9	0.4	4.89 ± 1.37mm	2.89 ± 1.39mm	2.33 ± 1.00mm	1.72 ± 0.67mm

Table B3. Modified Wound Healing Scale

MWHS	2wk	4wk	8wk	16wk
Control	1.67	1.00*	1.00	1.00
Experimental	2.00	1.56*	1.11	1.00

* Statistically significant between groups (Mann-Whitney U=12.0 p= 0.031)

Table B4. Patient Reported Outcomes (VAS)

Patient Reported Outcomes	Pain 2wk	Pain 4wk	Pain 8wk	Pain 16wk	Satisfaction
Control	27.0 ± 31.7	15.0 ± 20.5	14.7 ± 18.9	15.0 ± 20.5	99.0 ± 2.0*
Experimental	9.7 ± 8.1	4.8 ± 4.3	4.8 ± 8.3	4.8 ± 4.3	94.1 ± 7.2*

* Statistically significant between groups (Mann-Whitney U=9.5 p= 0.035)

Table B5. Keratinized Mucosal Width

KMW	Initial	Final (16-week)	Change
Control	6.00 ± 0.82mm	5.08 ± 0.66mm	-0.92 ± 1.02mm
Experimental	4.89 ± 1.37mm	4.61 ± 0.92	-0.28 ± 1.25mm

Table B6. Peri-implant Mucosal Thickness

KMW	Initial	Final (16-week)	Change
Control 1mm	2.33 ± 0.99mm	4.10 ± 0.93mm	1.70 ± 1.92mm
Control 3mm	2.50 ± 0.96mm	4.58 ± 1.53mm	2.08 ± 0.80mm*
Control 5mm	1.75 ± 0.85mm	2.58 ± 0.38mm	0.83 ± 0.75mm
Experimental 1mm	2.89 ± 1.39mm	3.00 ± 1.27mm	0.11 ± 1.65mm
Experimental 3mm	2.33 ± 1.00mm	3.17 ± 1.35mm	0.83 ± 1.37mm*
Experimental 5mm	1.72 ± 0.67mm	3.28 ± 0.91mm	1.56 ± 1.18mm

* Statistically significant between groups (Mann-Whitney U=910.0 p= 0.043)

APPENDIX C – Additional Forms

C.1. Informed Consent Document

Project Title: **COMPARISON OF TWO DIFFERENT SURGICAL APPROACHES TO INCREASE PERI-IMPLANT MUCOSA THICKNESS: A RANDOMIZED CONTROLLED CLINICAL TRIAL**

Principal Investigator: Dr. Gustavo Avila-Ortiz

Research Team Contact: **Dr. Avila-Ortiz, 319-335-7241**

Dr. Chris Hutton, 319-335-6775

Richard Barwacz, 319-335-6763

This consent form describes the research study to help you decide if you want to participate. This form provides important information about what you will be asked to do during the study, about the risks and benefits of the study, and about your rights as a research subject.

- If you have any questions about or do not understand something in this form, you should ask the research team for more information.
- You should discuss your participation with anyone you choose such as family or friends.
- Do not agree to participate in this study unless the research team has answered your questions and you decide that you want to be part of this study.

WHAT IS THE PURPOSE OF THIS STUDY?

This is a research study. We are inviting you to participate in this research study because you are about to get an implant at a site that has insufficient gum thickness, which may lead to a bad appearance. The purpose of this research study is to determine whether you will have a better outcome with a gum graft harvested from the roof of your mouth or a human tissue graft and how you perceive both discomfort and the esthetics of the graft during the healing period. In this study, we will be using Acellular Dermal Matrix (ADM) as the donor graft. ADM is a product derived from human skin that has been processed to eliminate any element that may lead to an allergic reaction or a cross infection (i.e. getting a disease from the donor). ADM works as a scaffold that allows your body to create a thicker gum tissue.

Before the baseline surgical intervention visit, you will be randomly assigned with the assistance of computer software to one of the following groups:

- Control Group: Implant placement with simultaneous gum graft obtained from the roof of your mouth

- Experimental Group: Implant placement with simultaneous ADM

HOW MANY PEOPLE WILL PARTICIPATE?

Approximately 20 people will take part in this study conducted by investigators at the University of Iowa College of Dentistry.

HOW LONG WILL I BE IN THIS STUDY?

If you agree to take part in this study, your involvement will last for approximately six months. There are 6 total study visits. The longest visit will be approximately 2 hours long, with the follow-up visits each taking about 30 minutes.

WHAT WILL HAPPEN DURING THIS STUDY?

Screening Visit

After reading and signing this informed consent form, you will complete a detailed medical and dental history form. We will review this form with you to ensure you can safely participate in the study. You will also have an oral exam to determine if you qualify for the study. We will take measurements of your gums to ensure you need a gum graft. We may take a dental x-ray if there is not a current x-ray on file to make sure the bone is stable. You will also have dental impressions (molds of your teeth), which are made in order to design and make a guide to record some of the research measurements. This visit will last about an hour and will take place in the Craniofacial Clinical Research Center at the College of Dentistry.

Baseline Surgical Visit

Within 8 weeks of the screening visit, you will have the gum graft in the Department of Periodontics at the College of Dentistry. Prior to this visit you will be randomized to one of two treatment groups:

1. If you are randomized to the **autologous graft group**, you will have a small portion of your own tissue harvested from the roof of your mouth. This graft will be secured in the area near your implant where you need a soft tissue graft.

2. If you are randomized to the **allograft group**, the study doctor will prepare and trim the commercial grafting material (ADM) and will fit and secure it in the area near your implant where you need a soft tissue graft.

This means that, whichever study procedure you receive, it will be determined purely by chance, like flipping a coin. You will have a 50/50 chance of receiving either of the two study procedures.

Before any surgical procedure begins, the study doctor will anesthetize the area. He will then make small incisions in the gums to expose a flap of gum tissue. The implant will be placed in your bone using a special drilling protocol. You will then either have your own tissue graft (you will have a slightly larger than pea-sized tissue graft harvested from the roof of your mouth) or the human tissue material (ADM) secured in the area near your implant where you need a gum graft depending on your study group. The graft and the flap will be secured with several stitches. The study doctor will make several measurements of your gums, which should not be painful. Photographs and videos of the study site may also be obtained. Before you leave, the study doctor will then provide you with post-operative instructions and will give you prescriptions for ibuprofen, an antibiotic, and an antiseptic mouth rinse. This visit will take approximately 2 hours.

Follow-up (2, 4, 8, and 16 weeks)

At each of the follow-up visits we will ask you what your average pain has been since the last visit and how you feel about the way your gum graft looks. You will receive an oral exam so that the study doctor can assess how your gums are healing. We will take photographs of your gum graft. At the 16 follow-up appointment, we will take measurements of your gums with a probe in addition to the above measurements and photos. You should not experience any pain during any of the measurements or photographs. Each of the follow-up visits should take about 30 minutes and will take place in the Craniofacial Clinical Research Center in the College of Dentistry.

Video Recording/Photographs

One aspect of this study involves making photographs of your gums. These photos are taken so that the study team can assess the final esthetics of your gum graft. Videos may be recorded in selective cases that are representative of the study procedures. No identifiable part of your face will be in the photographs and all photos and videos will only be identified by your unique study code. All the photos and video recordings obtained during the study will be securely stored in an encrypted computer, following UI computer security policies, and will be accessible only to study team members. These photos and video recordings will be kept to use in future presentations and publications.

WHAT ARE THE RISKS OF THIS STUDY?

You may experience one or more of the risks indicated below from being in this study. In addition to these, there may be other unknown risks, or risks that we did not anticipate, associated with being in this study.

You may experience some discomfort at the surgical visit during the anesthetic injections. You may have slight discomfort or pain during the first week of healing. If you are in the autologous graft group, you

may have additional pain with eating or drinking during the healing phase due to the additional graft site on the roof of your mouth.

There is always a risk of infection with any surgical procedure. There is a risk that the soft tissue graft in both treatment groups may not incorporate into the graft site and you would need a subsequent soft tissue graft if your surgeon recommends it.

Although the experimental material is tested for sterility and there is no reported case in dental literature of infection from the grafting material to be used in this study, as with the use of any graft material, there is the possibility of infection from its use.

If you are allergic to any of the elements contained in the grafting materials, you may experience a low blood pressure (hypotension) or in severe cases you could experience difficulty breathing (anaphylaxis).

There is also the risk that the graft may not incorporate and the grafting procedure may need to be repeated.

WHAT ARE THE BENEFITS OF THIS STUDY?

We don't know if you will benefit from being in this study. However, we hope that, in the future, other people might benefit from this study because we may gain valuable information about the effectiveness and esthetics of the two grafting materials.

WHAT OTHER TREATMENT OPTIONS ARE THERE?

Before you decide whether or not to be in this study, your doctor will discuss the other options that are available to you. Instead of being in this study, you could have the gum graft completed by a private periodontist, by another periodontist not affiliated with the study at the College of Dentistry, or you could not have the gum graft at all.

WILL IT COST ME ANYTHING TO BE IN THIS STUDY?

You will receive a discount for the implant placement procedure ($875). The regular price of implant placement is approximately $1,300 if performed by a resident at the College of Dentistry. The gum graft treatment will be paid in full for the study. Normally, both types of grafts cost $600 if performed by a resident dentist at the College of Dentistry. You will be responsible for the restorative costs related to the implant crown, which typically range between $1,250 and $1,500.

SERVICE	Regular patient care cost	Study patient cost
Implant placement	$1,300	$875
Gum graft	$600	No cost
Implant crown (restoration)	$1,250 - $1,500	$1,250 - $1,500

You and/or your medical/hospital insurance carrier will remain responsible for other regular medical care expenses.

WILL I BE PAID FOR PARTICIPATING?

You will be paid for participation in this research study. You will be paid $50 in two separate payments of $25, one at the 4-week visit and another one at the last follow-up visit (16 weeks). You will need to provide your address so that a check can be mailed to you.

WHO IS FUNDING THIS STUDY?

Biohorizons is funding this research study. This means that the University of Iowa is receiving payments from Biohorizons to support the activities that are required to conduct the study. No one on the research team will receive a direct payment or increase in salary from Biohorizons for conducting this study.

WHAT IF I AM INJURED AS A RESULT OF THIS STUDY?

- If you are injured or become ill from taking part in this study, medical treatment is available at the University of Iowa Hospitals and Clinics.
- The University of Iowa does not plan to provide free medical care or payment for treatment of any illness or injury resulting from this study unless it is the direct result of proven negligence by a University employee.
- If you experience a research-related illness or injury, you and/or your medical or hospital insurance carrier will be responsible for the cost of treatment.

WHAT ABOUT CONFIDENTIALITY?

We will keep your participation in this research study confidential to the extent permitted by law. However, it is possible that other people such as those indicated below may become aware of your participation in this study and may inspect and copy records pertaining to this research. Some of these records could contain information that personally identifies you.

- federal government regulatory agencies,
- the U.S. Food and Drug Administration and the sponsor, Biohorizons
- auditing departments of the University of Iowa, and
- the University of Iowa Institutional Review Board (a committee that reviews and approves

research studies)

To help protect your confidentiality, we will protect your privacy throughout the research study by doing the following; ID codes will be assigned to each subject, and no personal identifiers will be used for the ID code. The protected health information (PHI) gathered for the study will be limited to PHI that affects your inclusion/exclusion criteria and ongoing participation in the study. No other PHI will be collected. Only members of the research team will have access to your information. Records will be kept in locked cabinets within locked offices and password protected computers. Only 3 individuals will have access to these records: Ms. Lauren Hughes, Dr. Gustavo Avila, and Dr. Chris Hutton.

If we write a report or article about this study or share the study data set with others, we will do so in such a way that you cannot be directly identified.

A description of this clinical trial will be available on http://www.ClinicalTrials.gov, as required by U.S. Law. This website will not include information that can identify you. At most, the website will include a summary of the results. You can search this website at any time.

WILL MY HEALTH INFORMATION BE USED DURING THIS STUDY?

The Federal Health Insurance Portability and Accountability Act (HIPAA) requires the College of Dentistry to obtain your permission for the research team to access or create "protected health information" about you for purposes of this research study. Protected health information is information that personally identifies you and relates to your past, present, or future physical or mental health condition or care. We will access or create health information about you, as described in this document, for purposes of this research study and for your treatment. Once the College of Dentistry has disclosed your protected health information to us, it may no longer be protected by the Federal HIPAA privacy regulations, but we will continue to protect your confidentiality as described under "Confidentiality."

We may share your health information related to this study with other parties including federal government regulatory agencies, the University of Iowa Institutional Review Boards and support staff, and the sponsor, Biohorizons.

You cannot participate in this study unless you permit us to use your protected health information. If you choose *not* to allow us to use your protected health information, we will discuss any non-research alternatives available to you. Your decision will not affect your right to medical care that is not research-related. Your signature on this Consent Document authorizes the College of Dentistry to give us permission to use or create health information about you.

Although you may not be allowed to see study information until after this study is over, you may be given access to your health care records by contacting your health care provider. Your permission for us to access or create protected health information about you for purposes of this study has no expiration date.

You may withdraw your permission for us to use your health information for this research study by sending a written notice to Gustavo Avila, University of Iowa College of Dentistry, 801 Newton Rd., Iowa City, IA 52242. However, we may still use your health information that was collected before withdrawing your permission. Also, if we have sent your health information to a third party, such as the study sponsor, or we have removed your identifying information, it may not be possible to prevent its future use. You will receive a copy of this signed document.

IS BEING IN THIS STUDY VOLUNTARY?

Taking part in this research study is completely voluntary. You may choose not to take part at all. If you decide to be in this study, you may stop participating at any time. If you decide not to be in this study, or if you stop participating at any time, you won't be penalized or lose any benefits for which you otherwise qualify.

What if I Decide to Drop Out of the Study?

If you choose, you may leave the study at any time. If you leave the study before it is finished, there will be no penalty to you, and you will not lose any benefits to which you are otherwise entitled.

If you decide to leave the study early, we will ask you to contact one of the contact persons listed. It may be recommended that you return for post-operative appointments but not within the study.

Will I Receive New Information About the Study while Participating?

If we obtain any new information during this study that might affect your willingness to continue participating in the study, we'll promptly provide you with that information.

Can Someone Else End my Participation in this Study?

Under certain circumstances, the researchers or Biohorizons might decide to end your participation in this research study earlier than planned. This might happen because in our judgment it would not be safe for you to continue, because your condition has become worse, because you are or became pregnant, because funding for the research study has ended, because the sponsor has decided to stop the research, etc.

WHAT IF I HAVE QUESTIONS?

We encourage you to ask questions. If you have any questions about the research study itself or you experience a research-related injury, please contact: Dr. Gustavo Avila Ortiz, 319-335-7241, Dr. Chris Hutton, 319-335-6775, or Rick Barwacz, 319-335-6763.

If you have questions, concerns, or complaints about your rights as a research subject or about research related injury, please contact the Human Subjects Office, 105 Hardin Library for the Health Sciences, 600 Newton Rd, The University of Iowa, Iowa City, IA 52242-1098, (319) 335-6564, or e-mail irb@uiowa.edu. General information about being a research subject can be found by clicking "Info for Public" on the Human Subjects Office web site, http://hso.research.uiowa.edu/. To offer input about your experiences as a research subject or to speak to someone other than the research staff, call the Human Subjects Office at the number above.

This Informed Consent Document is not a contract. It is a written explanation of what will happen during the study if you decide to participate. You are not waiving any legal rights by signing this Informed Consent Document. Your signature indicates that this research study has been explained to you, that your questions have been answered, and that you agree to take part in this study. You will receive a copy of this form.

_____ _____

(Signature of Person who Consented) (Date)

Statement of Person Who Obtained Consent

I have discussed the above points with the subject or, where appropriate, with the subject's legally authorized representative. It is my opinion that the subject understands the risks, benefits, and procedures involved with participation in this research study.

_____ _____

(Signature of Person who Obtained Consent) (Date)

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