Apically positioned flap, free gingival graft and apically positioned flap with collagen matrix around dental implants: A randomized controlled trial.

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Abstract

Background: Dental implants have become very popular with success rate reaching around 98%. However, due to lack of anatomical structures, such as periodontal ligament, cementum and connective tissue, soft tissue around dental implants may be more susceptible to inflammation and breakdown. Therefore, attached keratinized mucosa (KM) will be more critical for dental implant and its prosthesis. There are different techniques used to augment keratinized mucosa. Studies have shown that free gingival graft, apically positioned flap and collagen matrix graft can be viable procedures to enhance soft tissue. However, given that all three procedures produce adequate amount of KM, no study focused on patients' comfort level during the procedure and healing. Also, there has been no randomized controlled trial that compared all three procedures in gaining of KM. **Aim**: To evaluate and compare patients' experience and effectiveness of free gingival graft (FGG), apically positioned flap (APF) with and without Mucograft around implants. The primary outcome was the level of patients' discomfort and pain during procedures and healing time. The secondary outcome was width of keratinized mucosa gain with esthetic evaluation. **Materials and Methods**: Subjects with lack of KM (<2mm) around dental implants were randomly assigned to one of the three groups; APF alone, FGG or APF with Mucograft, to receive soft tissue augmentation procedure. After the procedure, subjects received surveys regarding their experience during procedure and during healing period for pain, swelling, and bleeding. Amount of pain medication used (Tylenol#3) was also reported by subjects. KM was measured at 3 month follow-up visit. **Results**: Data for the primary outcome was collected from 28 subjects (9 APF, 7 FGG, 12Mucograft) and 24 subjects presented for 3 month follow-up keratinized mucosa measurement (7 APF, 7 FGG, 10 Mucograft). There was no difference in level of discomfort during the procedure between three groups. Mucograft group showed highest level of pain, swelling during healing and number of pain meds between the 3 groups ($P<0.05$). Gain of keratinized mucosa measured at 3 months showed no statistically significant difference. **Conclusion**: The results of this study suggest that soft tissue augmentation utilizing Mucograft may cause more discomfort than FGG or APF during the healing period. All three procedures provide adequate amount of keratinized mucosa.

Acknowledgement

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Introduction

As osseointegrated dental implants are becoming more common, the maintenance and complexity of their function and esthetics have become more challenging. While success of an endosseous dental implant depends on various physiologic and anatomical factors, gingival health around the implant plays the major role in maintenance of well-integrated dental implants by providing the primary defense against soft tissue breakdown which may lead to bone loss around dental implants. Studies that investigated anatomical structures of soft tissue around natural teeth and endosseous dental implants have shown that the anatomical structure around dental implants differ from natural teeth by lacking cementum, periodontal ligament and connective tissue attachment. Moreover, these anatomical differences are believed to make the soft tissue around dental implants more susceptible to inflammation and breakdown, which may affect the overall health of implants. [1, 2]

Despite the fact that need for keratinized mucosa remains controversial in maintaining gingival health, for the natural dentition, it is widely accepted among clinicians that keratinized attached gingiva plays a critical role in overall health of periodontium. Ericsson and Lindhe[3], in a dog study, showed the importance of keratinized tissue when treating periodontal disease using surgical techniques. Furthermore, Lang and Loe conducted a study on 32 dental students with 1406 tooth surfaces about gingival health and amount of keratinized mucosa. They had two groups of students, one with less than 2.0 mm of keratinized mucosa and the other group with more than 2.0 mm of keratinized mucosa, and showed teeth surfaces with less than 2.0 mm of keratinized mucosa presented with more clinical inflammation in gingival exudates, even though the subjects were in good plaque control.[4] The keratinized tissue (masticatory mucosa) differs

histologically from alveolar mucosa by containing more collagenous tissue, presenting as denser and less elastic texture than alveolar mucosa. Alveolar mucosa, which contains more blood vessels and less collagen structure than keratinized tissue, presents with less rigidity and density. As a result, when there is absence of keratinized tissue around dentition, the marginal tissue tends to be more susceptible to breakdown due to bacterial invasion and mechanical abrasion.

Concomitantly, the importance of keratinized mucosa around implants has been controversial for years. In many cases, the edentulous ridge for future dental implant may present with limited amount of keratinized mucosa for various reasons. Bone resorption after tooth loss may shift the mucogingival junction (MGJ) coronally and bone augmentation prior to implant placement may displace MGJ as well. [5] Many times, when a ridge preservation procedure is performed after a tooth extraction, soft tissue around the socket gets stretched in attempt to achieve primary closure or approximate the flap ends after bone graft material and barrier membrane insertion. As a result of this, the MGJ of the grafted area for a future implant placement displaces coronally. Thus, this coronally displaced MGJ will present with reduced corono-apical width of keratinized tissue band, which may result in lack of attached gingival around restored dental implants. Furthermore, there are several studies which indicate that lack of adequate keratinized gingival may arise as a critical problem for overall gingival health of dental implants. Bouri and Bissada showed in their study that increased keratinized mucosa around dental implants was associated with less alveolar bone loss and better soft tissue parameters which may reflect a better gingival health (e.g. Gingival index, Probing depth, Plaque index, etc.).[6] Also, the study by Zigdon and Machtei revealed that keratinized mucosa around implant does not only affect the clinical parameters but also immunological parameters. [7] In this study, width of keratinized mucosa had a negative correlation with level of recession around

dental implants. When dental implants had a wider band of keratinized mucosa, they were less likely to have gingival recession in future. More importantly, less periodontal pathogen was found around dental implants with wider keratinized mucosa. This is a very important finding because it implies that keratinized mucosa does not only provide mechanical protection but also plays a critical role at the immunological level as well. While reduced keratinized mucosa does not necessarily mediate adverse effects on the hygiene management and soft tissue health condition, the risk of increasing gingival recession and crestal bone loss is still present.[7,8] Other studies have addressed the more significant importance of keratinized mucosa around restorations and prostheses than around natural teeth regardless of oral hygiene status.[10,11] They have found a significant association between subgingival prostheses and gingival inflammation in areas with reduced keratinized mucosa in patients with less than ideal plaque control. Successfully integrated dental implants are most often restored with subgingival prostheses. Thus, these studies have great relevance to implant dentistry. Therefore, an inadequate amount of keratinized mucosa can be considered as a negative factor in the long term maintenance of marginal tissue around prostheses as well as dental implants. [12 -16]

To enhance the quality and the width of keratinized mucosa around implants, many surgical procedures have been applied and performed. [17] The surgical techniques include, but are not limited to, autogenous free gingival graft, apically positioning flap procedure and biomaterial grafting procedures (e.g. Mucograft, Geistlich).

Apically positioning flap (APF) is a relatively simple procedure that usually requires less chair time than other harvesting/grafting procedures. It requires no additional graft material. Therefore, patients need neither a donor site nor extra material. There are a few studies that researched direct impact of APF on soft tissue around natural teeth and dental implants.[18,19]

They showed that APF can be a viable option for enhancing width of keratinized mucosa.[20-22] Since 1950s, APF procedure has been suggested to be used for vestibuloplasty purpose prior to removable prostheses. It has been referred as a denudation technique or periosteal retention technique[23] because a wound was created to cause healing with more suitable soft tissue architecture, preferably keratinized mucosa, for future prostheses or other treatments. Carnio *et al.* [20-22] presented a series of papers in various journals with modified APF technique, which can effectively increase apico-coronal dimension of keratinized mucosa around teeth. In those studies, the authors extended the dimension of existing keratinized mucosa by making a horizontal incision slightly coronally to the mucogingival junction. This incision allowed the apical flap, which was displaced apically later, to have coronal margin of keratinized mucosa. The incised mucosa is dissected as a partial thickness flap leaving the periosteum intact on the surface of alveolar bone. The dissected flap is being displaced apically and secured, creating a wound surrounded by keratinized mucosa with exposed periosteum. Based on the study results, epithelium cells spread from surrounding keratinized mucosa into the wound during healing process. The wound created converted into attached gingival after healing, thus extending apico-coronal dimension of the existing band of keratinized mucosa. Furthermore, it was shown that superior patients' experience during and after the procedure was noted due to lack of harvesting and short chair time. Esthetic evaluation also revealed acceptable outcome in terms of soft tissue color and texture blending with surrounding areas. After all, the APF technique is suggested to be an effective method of gaining width of keratinized mucosa with comparable clinical outcome and more favorable patients' experience.

The free gingival graft procedure (FGG) has been performed widely in modern periodontology for its various application and great predictability. The current technique was

proposed by Sullivan and Atkins in 1969 and it has been widely used for treatment of mucogingival deformities. Studies have shown that FGG offers prolonged stability and integrity of keratinized mucosa with good predictability. In a retrospective long term evaluation study by Agudio *et al.* [24], FGG was shown to provide structural rigidity and physical defense against gingival recession and other mucogingival defects. They followed 224 surgical sites that were treated with FGG for lack of attached gingival for 10 to 25 years. And the authors found that those treated sites resulted in not only gain of keratinized mucosa but also significant reduction of recession depth. Due to its versatility, its application is not limited to increasing keratinized mucosa. FGG is also used for root coverage purposes and other pre-prosthetic vestibular preparation. Recently, the application of FGG has been extended to gingival augmentation around dental implants to protect periodontal health and eventually increase success of dental implants. [24-25] As mentioned above, during the course of implant treatment, mucogingival deformities occur often for various reasons. Many times, FGG is performed prior to restoring endosseous implants, especially during the 2nd stage implant surgery which is the uncovering of the implant fixture after osseointegration is complete. Despite the versatility and predictability of FGG, due to its donor tissue harvesting process, patients tend to complain of discomfort during the healing process. As standard, free gingival graft tissue is harvested from the palate of the same side as the recipient side to reduce discomfort and provide functionality to patients after the procedure. Since masticatory mucosa, which is the most superficial layer, is being harvested, the harvesting process leaves donor site with submucosa layer exposed to oral cavity. Often, when a patient needs to get a series of grafting procedures, the patient tend to decline the subsequent grafting procedures after the first one, due to the pain and discomfort on donor site.[23]

Periodontal dressing or palatal stent may be provided after procedure to reduce post operative discomfort. However, it is often difficult to control the discomfort completely.

Recently, keratinized tissue enhancement procedures using Mucograft have also been performed. These procedures are similar to FGG, however Mucograft is utilized as a substitute to autograft harvested from patients' palate. The major advantage of this type of procedure is no need for harvesting from patients, thus resulting in less discomfort for patients. Several studies have shown that Mucograft placed on apically positioned flap around both natural dentition and implants resulted in respectable gain of keratinized gingiva as well as good esthetic results. In the prospective pilot study by Nevins *et al.* in 2011[28], the authors conducted gingival augmentation procedures on patients' mandible. On one side, conventional autogenous gingival graft was performed and on the other side, Mucograft was used to augment soft tissue. Both sides showed significant amount of keratinized mucosa gain without statistical difference. Esthetically, Mucograft showed better tissue blending after healing. Histological analysis revealed that both sites showed similar tissue architecture with mature connective tissue and keratinized epithelium. However, this study was a pilot study case series and did not provide negative control without any grafting material to show actual benefit of utilizing Mucograft.

Sanz *et al.* in 2009 [29] performed a randomized prospective clinical trial comparing free connective tissue graft with Mucograft in gaining keratinized tissue around prostheses. The authors followed subjects for 6 months for evaluation of new keratinized tissue gain and esthetic evaluation. Both groups showed similar results in gaining of keratinized mucosa. The authors also conducted surveys for pain and dosage of analgesics following two procedures for each patient. The surveys indicated that patients had less discomfort and needed fewer analgesics following Mucograft procedures. As a result, the authors concluded that Mucograft application is

as effective a procedure as free connective tissue grafting for enhancing keratinized mucosa with significantly lower morbidity. Application of Mucograft is not only limited to enhancing keratinized mucosa. McGuire *et al.* [30], in a split-mouth randomized clinical trial, compared Mucograft with coronally advanced flap (CAF) to connective tissue with CAF in treating recession defects. The result showed that Mucograft may be an effective alternative to connective tissue graft when combined with CAF for recession treatment. Even with its clinical advantages, the biggest drawback of Mucograft application is the extra cost for the material. However, studies have shown that less morbidity and lower patients' discomfort level due to lack of graft harvesting maybe a more important factor in decision-making for many patients. [28-30]

Lee *et al.* [18] conducted a case series comparing three groups in achieving keratinized mucosa around dental implants at the time of 2nd stage. The case series had APF, APF with FGG and APF with collagen matrix groups. All three groups performed effectively achieving primary goal of gaining keratinized mucosa. However, the sample size of 9 subjects did not seem sufficient to provide enough evidence. Measurement methods and evaluation of outcome are also not very well described in this study.

Previous studies mentioned above may provide potential effectiveness of three surgical modalities proposed in this present study. However, most studies only compared two groups, Mucograft versus autograft. It will be difficult to determine whether there is an actual benefit from grafting the area with inadequate keratinized mucosa. And if there is any benefit, the differences in outcome among three modalities have to be evaluated systematically with proper methodology. So far, there has been no such study that evaluates and compares the relative effectiveness of all three modalities around dental implants in gaining keratinized mucosa with

standardized methodology and proper follow-up examinations. Therefore, adding a negative control group (APF alone) will greatly increase the validity of this present study.

Furthermore, most studies conducted previously focused only on gain of keratinized mucosa. However, as studies have suggested [7], only an 'adequate' amount of keratinized mucosa will be essential for periodontal health. Thus, once we achieve a certain level of gain, it will be very important to take patients' comfort and experience into consideration when we choose our treatment modality. Therefore, this present study measures pain and discomfort level from each procedure as primary outcomes and gain of keratinized mucosa and esthetic evaluation as secondary outcomes.

Specific Aims and Hypothesis

The objective of this study was to evaluate and compare apically positioned flap, free gingival graft and apically positioned flap with Mucograft, when performed for enhancement of keratinized mucosa around dental implants. The hypothesis of this study was that apically positioned flap will have less patients' discomfort and pain than free gingival graft.

This present study was the first randomized prospective controlled trial comparing FGG, APF and APF with allograft or xenograft material. The study not only compared effectiveness of three modalities, but it also evaluated overall patients' experience from each procedure. Comprehensive survey forms were developed to assess each patient's discomfort and pain level during the procedure and throughout the healing period. Chair time during procedure was

measured and survey forms were filled out by individual patients, including amount of analgesic drugs taken to cope with pain and discomfort post-operatively.

The primary outcome of this present study was patients' pain and discomfort level for each surgical modality. Since many studies have provided evidence for adequate effectiveness of all three techniques, it is more logical to thoroughly evaluate patients' experience because overall experience of a procedure plays a critical role when choosing treatment options. The secondary outcome, gain of keratinized mucosa and esthetics, was also evaluated with more refined standard methodology for better validity than previous studies.

Materials and methods

The study design for this research project is a prospective randomized controlled trial. Subjects were recruited from the Tufts University School of Dental Medicine Department of Periodontology clinic. The study was approved by the Tufts Health Sciences Campus Institutional Review Board.

Data collection for the primary outcome and esthetic evaluation were done through surveys from the subjects and independent evaluators. For the secondary outcome, data collection was done by clinical measurements. All subjects received two staged implant surgery, surgical placement of implant fixture and uncovering of fixture after osseointegration. At the time of the 2nd stage, patients were evaluated for qualification to participate in the study based on inclusion and exclusion criteria (see below). Qualified patients were invited to participate in the

study. After informed consent was provided, subjects were randomly assigned to one of three surgical procedure groups; apically positioning flap without Mucograft, free gingival graft, or apically positioning flap with Mucograft. The randomization process was done by a sequence randomization system on the website www.random.org.

Based on the sample size calculation (nQuery Advisor, 7.0), the initial planned sample size was 16 subjects in each group, for a total of 48 subjects in the study. The calculation was based on 2 point difference in VAS from the pain survey, assuming a common standard deviation of 2.0, setting α=0.05and requiring 80% power.

The **inclusion criteria** were:

1) Two staged implant procedures.

2) Inadequate keratinized tissue (less than 2mm of buccal keratinized mucosa prior to the time of 2nd stage surgery). [4]

3) Adequate depth of vestibule (>7 mm from the crest of ridge) to accommodate gain of keratinized mucosa width.

The **exclusion criteria** were:

1) Absence of keratinized mucosa on the area of implant.

2) Systemic conditions which may interfere with soft tissue healing, e.g. autoimmune diseases

3) Any uncontrolled local or systemic disease that might contraindicate invasive periodontal surgery.

4) Previous gingival grafting procedure on the area.

5) Smoking (> one pack a week)

The **withdrawal/termination criteria** were:

1) Subjects who failed to present for the 6-month follow-up appointment. (Forthe secondary outcome)

2) Subjects who failed to present to more than two post-operative appointments over the course of data collection.

Pre-operative preparation

All subjects were screened as proposed in the protocol using inclusion and exclusion criteria. During the visit 1, informed consent forms were signed, comprehensive periodontal exams (PI, GI and PD), oral hygiene instruction (OHI), and plaque control were done. Alginate impressions were taken for the arch that was being treated to fabricate measuring stent. Any existing inflammatory periodontal disease (gingivitis and periodontitis) was treated prior to performing the assigned surgical procedure. Any parafunctional habits that might have damaged soft tissue were addressed as well. Within 2 weeks prior to the surgical procedure, plaque control was re-evaluated and prophylaxis was given to provide the optimal environment for surgery.

Measurement stent was fabricated for each subject using blue ‘Triad’ denture material. The stent was to fit into the area of interest. A groove was also made on the stent for insertion of a periodontal probe. This stent provides a reproducible location and angulations for each measurement along the follow-up time. All measurements were done by a co-investigating faculty member who did not supervise the surgical procedure.

Although the stents were fabricated prior to each procedure for each patient, the groove on the stent was marked right after the procedure, at the midbuccal line of healing abutment for the precise location of measurement.

Subjects were given instructions to complete the surveys collecting information during the procedure and during the healing period.

Surgical procedures

After proper preparation, the randomly assigned surgical procedures were performed. To ensure consistency in the level of surgical expertise, the surgical procedure was performed by the MS candidate resident under the supervision of principal investigator, Dr. Yong Hur or co-investigator, Dr. Yumi Ogata. For all surgical procedures, baseline measurements which include plaque index, gingival index and probing depth were completed.

All procedures were performed under local anesthetics. Chair time for each procedure was also measured. All three surgical procedures included 7mm width of recipient bed extended apically to initial horizontal incision to provide standardized baseline dimension.

<u>Apically positioned flap</u>:

- Center of implant fixtures was located.
- Crestal incision through the center of implant fixture was made.
- Implant fixture was uncovered and healing abutment was inserted.

- Horizontal incision at 0.5mm coronal to buccal mucogingival junction was made 1 tooth mesial to 1 tooth distal to the treating area.
- No vertical incision was made
- Gingiva coronal to horizontal incision remained intact
- Partial thickness flap was prepared and displaced apically 7mm from the horizontal incision.
- Partial thickness flap was secured with 5-0 Chromic gut as simple interrupted.
- Prepared surgical wound was measured apico-coronally and mesio-distally.

Free gingival graft

- Center of implant fixtures was located.
- Crestal incision through the center of implant fixture was made.
- Implant fixture was uncovered and healing abutment was inserted.
- Horizontal incision at 0.5mm coronal to buccal mucogingival junction was made 1 tooth mesial to 1 tooth distal to the treating area.
- Vertical incisions were not made on both mesial and distal side of recipient.
- Gingiva coronal to horizontal incision remained intact
- Partial thickness flap was prepared and displaced apically 7mm from the horizontal incision.
- Partial thickness flap was secured with 5-0 Chromic gut as simple interrupted.

- Prepared recipient bed was measured apico-coronally and mesio-distally.
- Masticatory mucosa from palate of the treating side (Right or Left) was harvested according to the size measured from the recipient bed with width of 5mm at the line of measurement.
- Harvested graft material was transplanted on the recipient bed, lined with the initial horizontal incision line, by simple interrupted sutures using 5-0 Monocryl suture.

Apically positioned flap with Mucograft

- Center of implant fixtures was located.
- Crestal incision through the center of implant fixture was made.
- Implant fixture was uncovered and healing abutment was inserted.
- Horizontal incision at 0.5mm coronal to buccal mucogingival junction was made 1 tooth mesial to 1 tooth distal to the treating area.
- No vertical incision was made
- Gingiva coronal to horizontal incision remained intact
- Partial thickness flap was prepared and displaced apically 7mm from the horizontal incision.
- Partial thickness flap was secured with 5-0 Chromic gut as simple interrupted.
- Prepared recipient bed was measured apico-coronally and mesio-distally.

- Mucograft material was prepared according to the manufacturer's instruction and trimmed as the size measured from the recipient.
- Mucograft was placed and secured on the recipient bed with 5-0 Monocryl as simple interrupted.

Post-operative care

A periodontal dressing, Coe-Pak, was applied to all surgical sites. Ice pack and analgesics (Ibuprofen 800mg) were provided. Post operational instruction regarding plaque control and diet were given to subjects. After all clinical procedures, subjects were asked to complete a survey form related to the experience during the procedure. Once the procedure was completed, subjects were given the medication log for Tylenol #3, along with instructions to fill out.

Subjects were given Ibuprofen 800mg TID for 3 days for basic pain medication. Tylenol #3 was also prescribed and subjects were advised to take it as needed for pain. The number of Tylenol #3 pills was recorded in the pain medication log by the subject. Azithromycin 250mg was prescribed to take twice a day for the 1st day and once a day for day 2 to day 5. Subjects were instructed to rinse with 0.12% Peridex for 10 days to substitute tooth brushing. All sutures were removed at 10 days.

Subject Surveys

The survey form related to level of discomfort during the procedure was marked by the subject with scale from 0 to 10.

The survey related to the healing period had subject rate the level of post-operative pain, swelling and bleeding to be marked with a scale from 0 to 10. The survey form also included the amount of analgesics (Tylenol #3) the subject took by day and time in one hour interval. The survey forms are attached in the appendix.

Follow up visits

There were follow up visits at 10 days, 3 weeks, 6 weeks, 3 months and 6 months. The followings were completed for each follow up visit.

- 10 days: Survey form collection, periodontal dressing removal, suture removal, evaluation of healing, professional cleaning of the surgical site, oral hygiene instruction as needed, and administration of the during the healing period survey.
- 3 weeks: Evaluation of healing, and professional cleaning of the surgical site
- 6 weeks: Evaluation of healing and prophylaxis, reinforcement of oral hygiene instructions.
- 3 months: Measurement of periodontal parameters (PI, GI and PD), prophylaxis, reinforcement of oral hygiene instructions, measurement of keratinized mucosa, intraoral photographs.
- 6 months: Measurement of periodontal parameters (PI, GI and PD), prophylaxis, reinforcement of oral hygiene instructions, measurement of keratinized mucosa, intraoral photographs.

Esthetic evaluation

Esthetic evaluation will be done by two independent examiners, Department of Periodontology faculty members, using the esthetic evaluation survey form. The examiners will be provided with intraoral photographs taken at 6 months for each subject to use for the esthetic evaluation. The survey form will have categories for level of ginginval margin, marginal tissue contour, soft tissue texture, mucogingival junction alignment and gingival color with scale of 0 to 10 to be checked by the two examiners. The survey form is attached in appendix.

Keratinized mucosa measurement

Baseline measurement of surgical wound width plus existing keratinized mucosa on buccal of healing abutment were the reference for change in keratinized mucosa width over the course of time. Right after the procedure had been completed, using the measurement stent and periodontal probe, the width from the apical point of surgical wound to the crest of gingiva at midbuccal line of healing abutment was measured.

For 3 month and 6 month follow up, the mucogingival junction and attached mucosa were identified clinically. The width of keratinized mucosa was measured from the mucogingival junction to the crest of gingival using the same stent to reproduce the location and angulations of the measurement. At the time of each measurement, intraoral photographs were also taken with standardized magnification and angle. Digital images, calibrated with a periodontal probe inserted on the stent groove when photos were taken, were analyzed using ‘Image J’ software to confirm measurements.

Compensation

Participating subjects received all surgical and related procedures free of charge. Subjects also received a gift card of $20 when presented for both visit 4 and visit 5 for the measurement of keratinized mucosa and intraoral photos.

Statistical analysis

For the primary outcome, pain, data were obtained from self-reported surveys using a 10-point VAS scale. The gain of keratinized mucosa measured by periodontal probe in millimeters with stent was used as data for secondary outcomes.

For all variables, normality was assessed using the Kolmogorov-Smirnov test. If the assumptions of normality hold, then means and standard deviations were reported and the relationship between treatment arm and the outcome was tested using one-way ANOVA. If the assumptions of normality did not hold, then medians and interquartile ranges were reported and relationships were tested using the Kruskal-Wallis test. If the initial analyses were significant, then the *post hoc* pairwise comparisons were made using either independent-sample t-tests or Mann-Whitney U-tests. When patients were lost to follow-up, their information was excluded from the analyses. All p-values less than 0.05 were considered statistically significant. Analyses were performed using IBM SPSS, version 21.

The data collected for the primary outcome did not meet the assumptions of normality. Therefore, Kruskal-Wallis test was used to evaluate the differences between three groups. For variables that showed statistically significant differences between groups, Mann-Whitney U tests were performed between groups as *post hoc* pairwise comparison. Chair times after each

procedure were also analyzed between groups using One-way ANOVA for statistical significance. Bivariate comparison tests were also performed using Pearson Correlation to see if there is correlation between chair time and each variables of the primary outcome that measured level of subjects' experience during and after the surgical procedures. All variables for the secondary outcome showed normal distribution of data. Therefore, One-way ANOVA was performed to test the significance of differences among three groups.

Result

A total of 30 subjects with mean (standard deviation [SD]) age of 57 (15.3) years were enrolled in this study. Among those, 16 were male and 14 were female. Before the surgical procedures, two subjects dropped from the study, one had scheduling issue with procedure appointments and the other subjects had failed implant at the time of procedure. During Visit 1, baseline periodontal parameters, including plaque free index, bleeding index (BI) and probing depth, as well as initial width of keratinized mucosa were measured in visit 1 (Table 1). 5 subjects (2 in APF group, 1 in FGG group, 2 in APF with Mucograft group) who were fully edentulous were excluded from periodontal measurements (PI, BI, and PD). The periodontal parameters did not show statistically significant differences between groups. Among 28 subjects who received surgical procedure in visit 2, 9 received apically positioned flap, 7 received free gingival graft and 12 received apically positioned flap with Mucograft. All surgical procedures were performed following the established and approved protocol.

Subjects' experience during and after surgical procedures

Survey data from subjects' intra-operative discomfort level (0-10) showed no statistically significant differences among the three groups (p=0.344). For the variables measured for subjects' experience during the healing period, there was statistically significant differences among the three groups in pain (p= 0.036), swelling (p= 0.044) and number of Tylenol #3 (p= 0.036). For the pain during the healing, the APF with Mucograft group showed the highest median score (3.5) with IQR of (1.75, 6.50) while APF group showed the lowest median score (1.0) with IQR of (0.0, 2.0). For the swelling during the healing, the APF with Mucograft group showed the highest median score (3.5) with IQR of (1.75, 6.50) while APF group showed the lowest median score (1.0) with IQR of (0.0, 3.0). And for the number of Tylenol #3 taken during the healing, the APF with Mucograft group showed the highest median score (0.5) with IQR of (0, 9) while APF group showed the lowest median score (0) with IQR of (0.0, 0.0) (Table 2).

Mann-Whitney pairwise *post-hoc* test was performed for the three variables that showed statistically significant differences between groups. It was observed that statistically significant differences lie between apically positioned flap group and APF with Mucograft group in all three variables; pain (p= 0.021), swelling (p= 0.016), Tylenol #3 (p= 0.016) (Table 3).

Chair time and subjects' experience

Chair time was measured for all 28 surgical procedures and there was a statistical difference in chair time among the three groups (p= 0.002). APF with Mucograft group showed the longest mean [SD] chair time (72.4 [6.2] min) followed by free gingival graft group (63.0

[21.8] min) and apically positioned flap group (45.1 [8.1] min) (Table 4). When correlations were looked at using Pearson Correlation, the variables of primary outcome did not show any correlation with the chair time, except the level of bleeding during the healing period which showed a weak positive correlation with the chair time (r=0.384, p= 0.044).

Measurement of keratinized mucosa

Twenty-four subjects presented to clinic for follow up visits at 3 month from the surgical procedure (visit4). One subject became pregnant and dropped out of the study and the other 3 subjects were lost to follow-up. The collected data in Table 5 shows that free gingival group showed the mean (SD) of 4.6 (1.2) mm for the total keratinized mucosa at visit 4 (2-3 month post-op) with IQR of (3.81, 5.17). The apically positioned flap with Mucograft group showed the mean (SD) of 3.9 (1.1) mm with IQR of (2.93, 4.87). And the apically positioned flap group showed the mean (SD) of 3.9 (1.7) mm with IQR of (2.53, 5.86). However, the differences were not statistically significant (p=0.548). The same pattern was also observed when the keratinized mucosa gained from each procedure was compared. The free gingival graft group showed the mean (SD) of 3.2 (1.2) mm with IQR of (3.00, 3.73), followed by the apically positioned flap with Mucograft group with mean (SD) of 3.1 (1.1) mm and IQR of (2.32, 4.01). The mean (SD) of 2.2(1.6) mm was observed in apically positioned flap group with IQR of (0.51, 3.89). And the differences were not statistically significant (p= 0.301).

The initial amount of keratinized mucosa showed statistically significant difference among three groups, with apically positioned flap group having the most amount of initial

keratinized mucosa. However, there was no statistically significant differences among groups when the keratinized mucosa measured at 3 months and the increased amount of keratinized mucosa were analyzed.

Discussion

This randomized controlled trial compared apically positioned flap, free gingival graft and apically positioned flap with Mucograft on clinical and pain/discomfort outcomes. We found that overall discomfort level during the healing period was the highest in APF with Mucograft group, followed by free gingival group and apically positioned flap group. At 3 months after the procedure, no difference in gain of keratinized mucosa gain was observed in all three groups. However, the results may have been influenced by the small sample size and also unbalanced distribution of the subject groups.

The only other study conducted with three groups (FGG, APF and APF with collagen membrane) was by Lee *et al.* [18] and it was a case series of 9 patients. With outcomes on both individual subject's experience and effectiveness of each surgical procedure, our study will be able to provide the clinicians with more information and evidence when choosing a surgical technique to enhance keratinized mucosa around dental implants. By having subject's pain and discomfort level as the primary outcome, this study was the first study that systematically evaluate overall patient experience after three commonly performed surgical procedures with the same goal of attaining newly formed keratinized mucosa. Based on experience and personal

perception, clinicians tend to believe that free gingival autograft procedures may involve more morbidity and discomfort due to the secondary surgical site for harvesting.

Subjects who had free gingival graft procedure did not express more discomfort during the procedure and this is contrary to many experts' opinion and results from previous studies that suggest higher level of discomfort after FGG due to additional surgical site for harvesting epithelium tissue from palate which involves not only incisions and sutures but also local anesthetic injections. The palate is known to be the most uncomfortable area for patients to get injected due to its restricted anatomical space and presence of masticatory mucosa that may inhibit absorption of topical benzocaine anesthetics, which is commonly applied on soft tissue epithelium before the injection to reduce patients' discomfort. [31] For this particular result, small sample size and uneven distribution of subjects may need to be taken into consideration for interpretation.

More interestingly, the results from this study suggest that subjects experienced more discomfort during the initial healing period after surgical procedure with Mucograft. This result is contrary to the outcomes of many studies that compared Mucograft versus autograft (free gingival graft or connective tissue graft) in recession coverage or keratinized mucosa augmentation. Most previous studies concluded that patients had significantly lower morbidity and discomfort level when treated with Mucograft. Sanz *et al.*[29] in 2009 and McGuire in 2010 [27] analyzed subjects' discomfort and morbidity level using VAS patients survey and concluded that Mucograft provides comparable outcome as their control group (autograft) and significantly lower morbidity and discomfort. In their explanation, lower discomfort and morbidity level have been mainly attributed to no need for harvesting and 2nd surgical site. It may seem reasonable to draw the assumption or conclusion that procedure with Mucograft will definitely reduce the level

of discomfort and morbidity without harvesting soft tissue from patients' palate, yet this present study shows that it may not be the case. Mann-Whitney pairwise test revealed that the significant difference lies betweenapically positioned flap group and Mucograft group. The difference between apically positioned flap and free gingival graft was not statistically significant. This suggests that, with proper post operative management and surgeon's skill, it is possible to keep the post operative discomfort level similar to that of a procedure without harvesting from the palate.

As the significant difference is between apically positioned flap group and Mucograft group, it can be speculated that Mucograft may interfere with healing process of the surgical wound due to its foreign nature (porcine xenograft). Since the material is not from subjects' body, it is expected to have rejection-like response during its adaptation when grafted on subjects' oral cavity, although the graft material is chemically processed to be sterile and hypoallergenic. This may have led to significantly higher level of post operative pain, swelling and amount of Tylenol #3.

Also, there was statistically significant difference in chair time among the three procedures, apically positioned flap being the shortest procedure and APF with Mucograft being the longest procedure. But when individual variables for subjects' experience were compared with chair time, longer chair time did not translate into higher scores in subjects' discomfort level.

Measurement of keratinized mucosa was done at visit 4 for 3 month follow-up using the stent fabricated with 'Triad' custom tray material. All three groups showed adequate and comparable amount of keratinized mucosa, ranging from 3mm to 4mm in mean value. Total

amount of keratinized mucosa measured at 3 month post-op was the highest in free gingival graft group, followed by the apically positioned flap with Mucograft group and the apically positioned flap group. The same pattern appears when keratinized mucosa at 3 month post-op was compared to the initial keratinized mucosa amount. However the difference among 3 groups was not statistically significant. This outcome is very similar to the results of previous studies that showed comparable performance and efficacy of Mucograft when used to cover recession or increase zone of keratinized mucosa. [18, 28-30] Although free gingival graft did not show the highest gain in keratinized mucosa, based on clinical observation, this procedure tends to produce more consistent and predictable outcome in terms of healing and quality of soft tissue than procedure with Mucograft. It may be said that Mucograft can be an alternative option for autograft such as free gingival graft or connective tissue graft, yet the procedure using Mucograft could be more case as well as technique sensitive. However, further studies are needed to confirm this observation.

Limitations

One of the limitations of this study may be its limited sample size. There were 28 subjects for primary outcome data and 24 subjects for secondary outcome data. Although statistically significant results were obtained for the primary outcome, there was no statistically significant result for the secondary outcome. This result may have been significant if there were bigger sample size. The other limitation of study population is that there was uneven distribution of subjects in 3 groups; 9 subjects for APF group, 7 for FGG group and 12 for APF with

Mucograft group. This uneven distribution of subjects may have played a role in the lack of significance in some comparisons.

Each subject may have different response to treatment, procedure and material in terms of discomfort level, healing process and clinical outcome. Therefore, for an ideal comparison of different surgical procedures, it would be more desirable to have all three types of procedures performed in each subject, and compare subjects' intraoperative and postoperative discomfort level as well as clinical outcome in terms of increased keratinized mucosa width.

This present study has measurement of keratinized mucosa for up to 3 month follow-up. For more comprehensive and thorough evaluation of clinical outcome from each procedure, longer follow-up such as 6-month, 2-year or 3-year period of time will be necessary. Soft tissue remodels throughout the time after a surgical procedure. Though, 6 months evaluation for the subjects may provide initial results and healing of each procedure, it will be also interesting to follow the subjects for a longer period of time, 1 to 3 years, and re-evaluate surgical areas how the soft tissue remodels and stays after different procedures.

Conclusion

The results of this study suggest that there is no difference in patients' level of discomfort during the surgical procedure when apically positioned flap, free gingival graft and APF with Mucograft were compared. However, for the initial healing period (7-10 days), apically positioned flap with Mucograft causes significantly more postoperative discomfort in terms of pain and swelling when compared to apically positioned flap and free gingival graft. Also, during

the course of this study, subjects needed more pain medication (Tylenol #3) after apically positioned flap with Mucograft than other two types of procedures. Also, the result of this study shows that chair time during the surgical procedure does not strongly correlate with patients' pain or discomfort level.

All three procedures produces adequate amount of keratinized mucosa when measured at 3 month after the procedure, but there was no statistically significant differences between the three groups. However, further study with bigger sample size will be necessary to confirm these results.

Appendix I: Tables
Appendix II: Clinical photos
Appendix III: Sample survey for pain and discomfort
Appendix IV: Subjects flow chart

Table 1.Subjects' demographic and baseline periodontal parameters.

		Apically Positioned Flap	Free Gingival Graft	APF with Mucograft	p-value
N		9	7	12	
Age (mean +/- SD)		60.2 ±17.196	74.7 ±12.623	50.3 ±14.511	
Gender (M/F)		3/6	7/0	5/7	
PI (%)	Mean +/- SD	76.00 ±20.363	87.83 ±5.845	77.30 ±14.930	.328
	IQR	(73.0, 89.0)	(83.0, 92.5)	(75.5, 87.0)	
BI (%)	Mean +/- SD	41.71 ±17.299	19.17 ±15.012	37.60 ±21.537	.097
	IQR	(30.0, 52.0)	(4.8, 34.8)	(20.5, 50.5)	
PD >4mm (%)	Mean +/- SD	4.71 ±4.608	1.33 ±2.338	8.00 ±7.760	.113
	IQR	(2.0, 7.0)	(0.0, 2.3)	(2.8, 11.3)	
KM (mm)	Mean +/- SD	1.62 ± 0.454	1.44 ± 0.551	0.88 ± 0.511	.006
	IQR	(1.74,2.01)	(0.80, 2.01)	(0.45, 1.17)	

For PI, BI and PD, fully edentulous patients were excluded. (N= 7, 6, 10)

Table 2.Descriptive data for primary outcome: during the procedure and during the healing period experience. (VAS 0-10)

	Apically positioned flap (N=9)		Free gingival graft (N=7)		APF with Mucograft (N=12)		
	Median	IQR	Median	IQR	Median	IQR	p-value
Discomfort during procedure	1.0	(0,3)	2.0	(1,5)	1.0	(0, 2.3)	0.344
Pain during healing	1.0	(0,2)	1.0	(0,4)	3.5	(1.8, 6.5)	0.036
Swelling During Healing	1.0	(0,3)	2.0	(0,3)	3.5	(1.8, 6.5)	0.044
Bleeding During Healing	0.0	(0,0)	0.0	(0,1)	0.0	(0, 2.3)	0.108
Number of Tylenol #3	0.0	(0,0)	0.0	(0,1)	0.5	(0,9)	0.044

Table 3. Mann-Whitney U test for statistically significant primary outcome differences.

	APF vs FGG	APF vs APF with Mucograft	FGG vs APF with Mucograft
Pain During Healing	.956	.021	.054
Swelling During Healing	.869	.016	.096
Number of Tylenol #3	.098	.016	.278

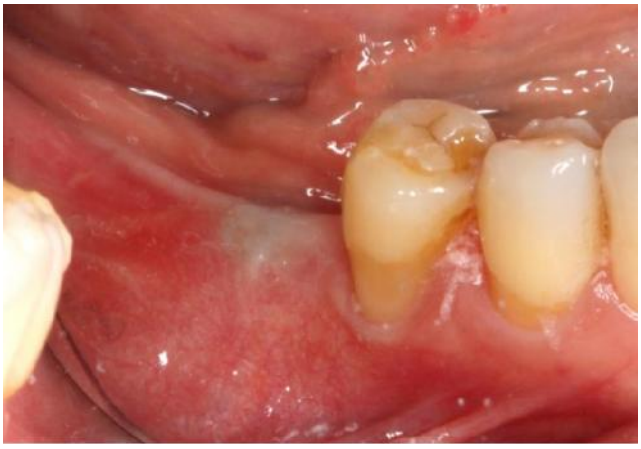
Table 4. Chair time among three groups and Bivariate (Pearson) correlation with variables for subjects' discomfort during and after each procedure.

	Apically Positioned Flap N = 9	Free Gingival Graft N = 7	APF with Mucograft N = 12	p-value
Minutes Mean (SD)	45.1 ±8.08	63.0 ±21.75	72.4 ±16.17	
IQR	(39.5, 51.5)	(40.0, 79.0)	(70.5, 75.0)	.002
Bivariate Correlation				
Discomfort During Procedure	r= 0.144, p=0.464			
Pain During Healing	r=0.112, p=0.570			
Swelling During Healing	r=0.017, p=0.930			
Bleeding During Healing	r=0.384, p=0.044			
Number of Tylenol #3	r=0.021, p=0.914			

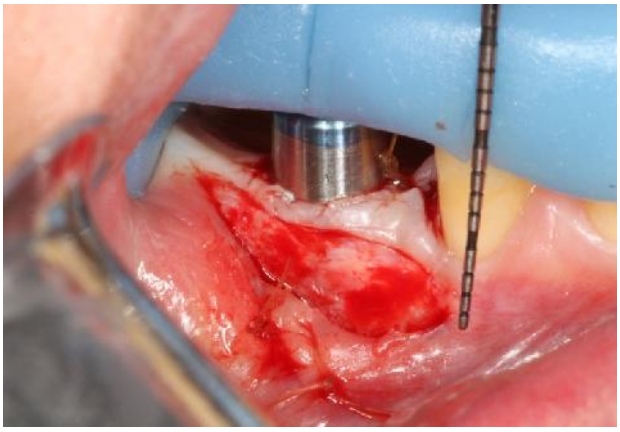
Table 5. Descriptive data for secondary outcome: Measurement of keratinized mucosa at 3 months post-op and gain of keratinized mucosa.

	Apically positioned flap (N=7)		Free gingival graft (N=7)		APF with Mucograft (N=10)		
(mm)	Mean (SD)	IQR	Mean (SD)	IQR	Mean (SD)	IQR	p-value
Keratinized Mucosa at 3 mos	3.94 ± 1.667	(2.53,5.86)	4.63 ± 1.232	(3.81, 5.17)	3.99 ± 1.113	(2.93, 4.87)	0.548
Increased KM at 3 mos	2.23 ± 1.599	(0.51, 3.89)	3.20 ± 1.226	(3.00, 3.73)	3.12 ± 1.080	(2.32, 4.01)	0.301

Photographs: apically positioned flap procedure

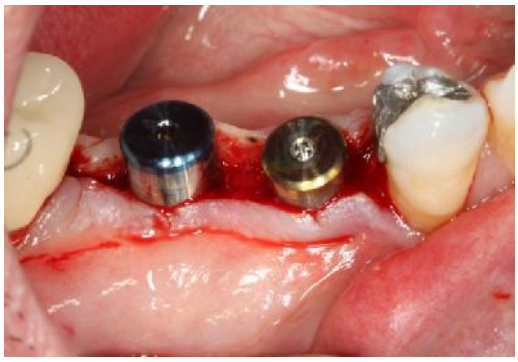


Pre-Op, note lack of buccal keratinized tissue

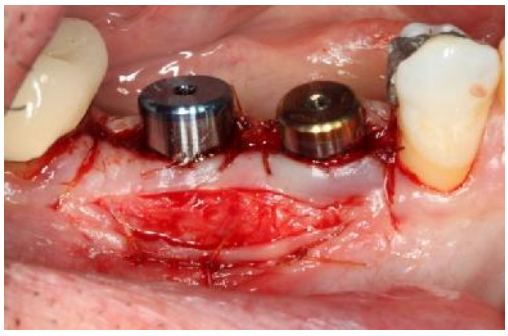


Post-Op, Healing abutment placed, APF completed exposing periosteum

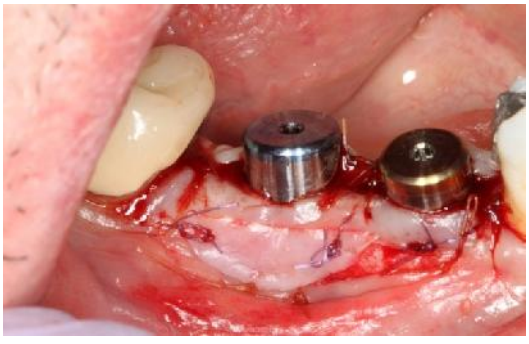
Photographs: Free gingival graft procedure.



Note lack of keratinized mucosa on buccal side

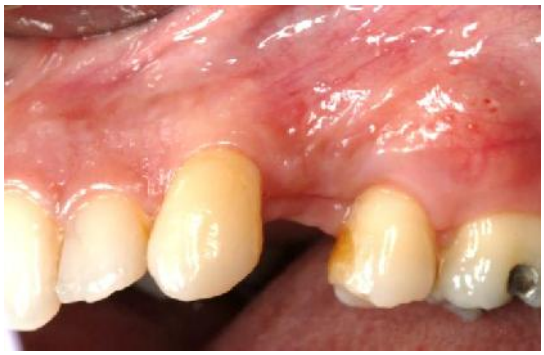


Recipient bed prepared

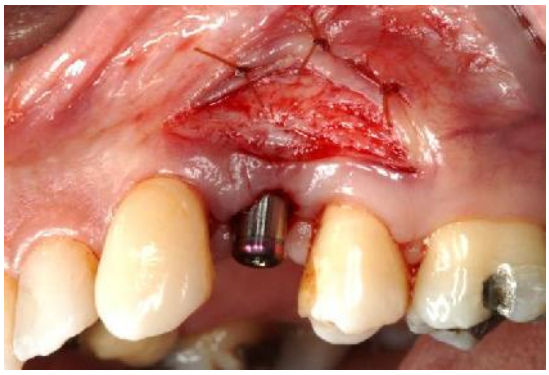


Harvested graft placed on recipient site

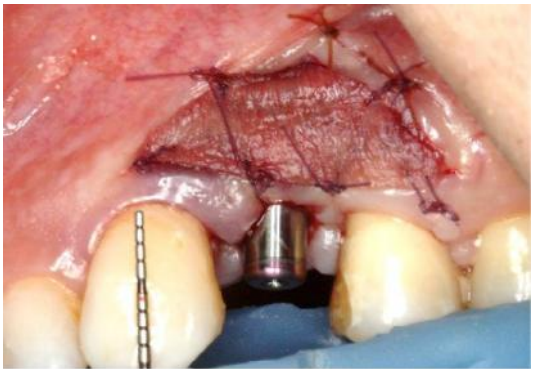
Photographs: Apically positioned flap with Mucograft procedure.



Pre-Op. Note lack of keratinized mucosa

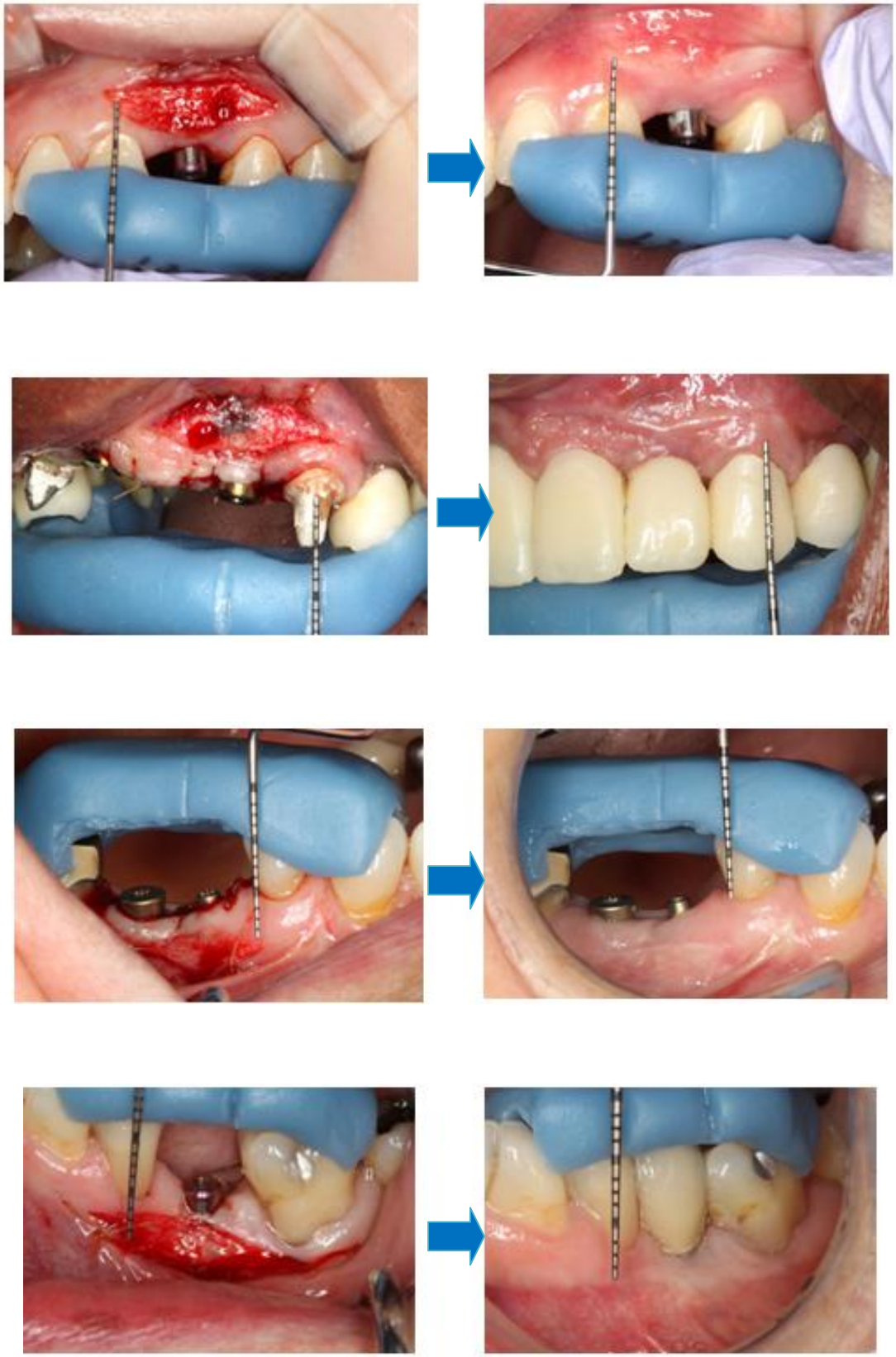


Recipient bed prepared

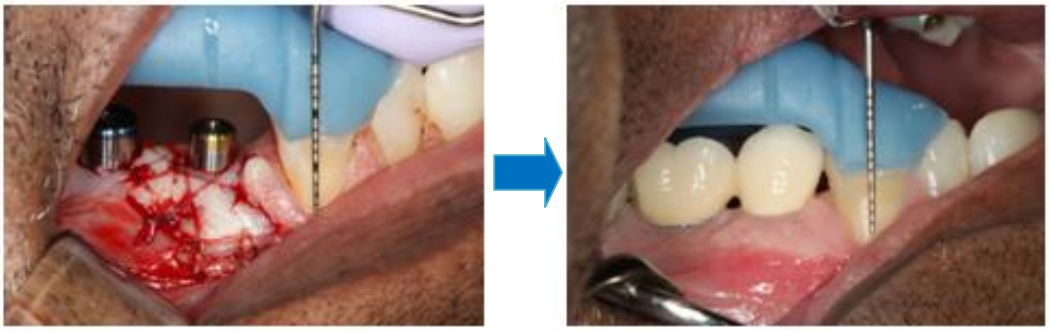


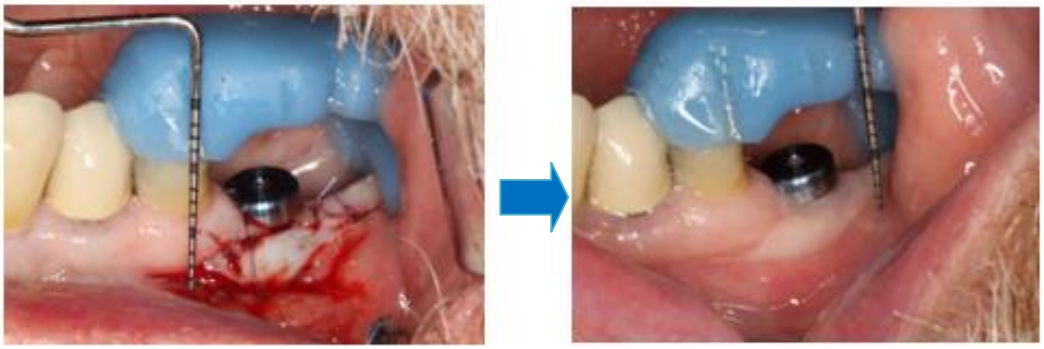
Mucograft placed on recipient site

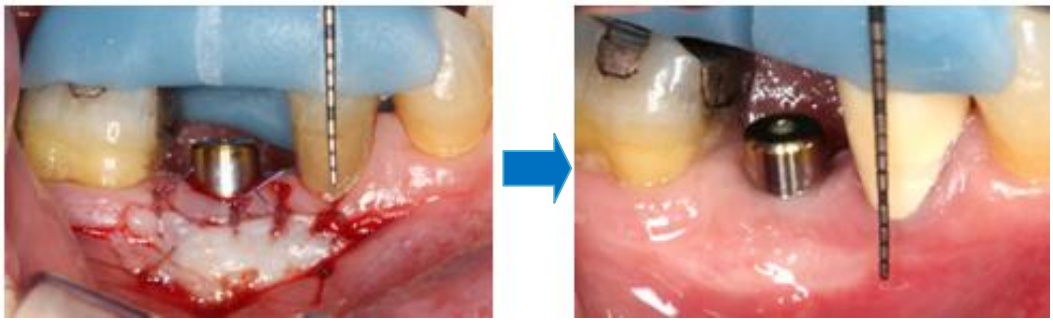
Measurement of keratinized mucosa at 3 month follow- up– Apically positioned flap

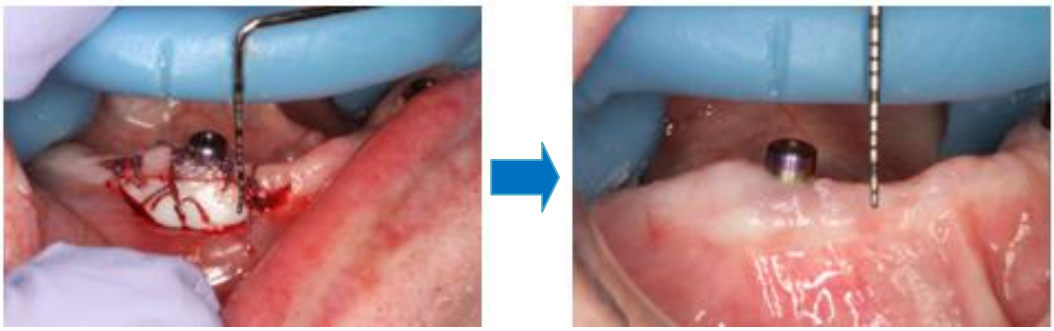


Measurement of keratinized mucosa at 3 month follow- up –Free gingival graft

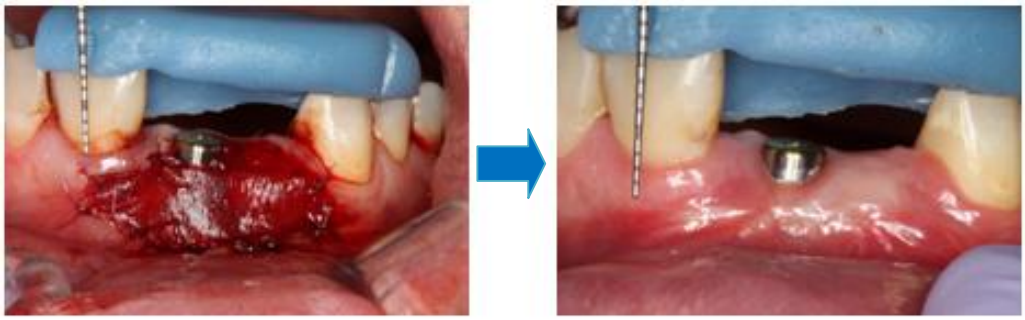


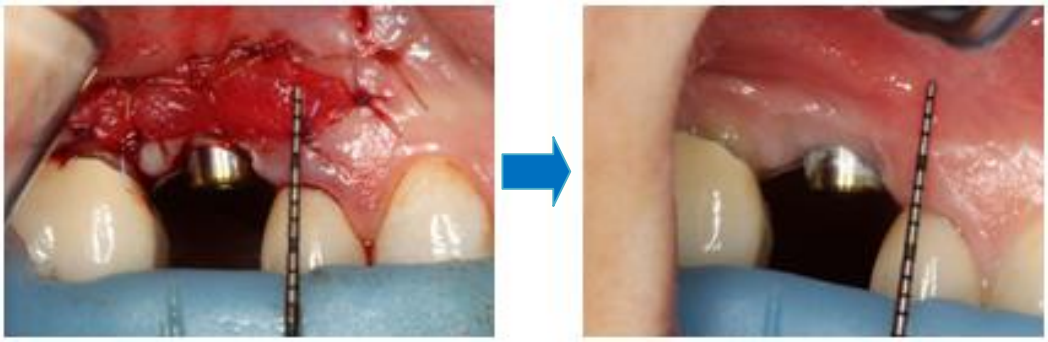


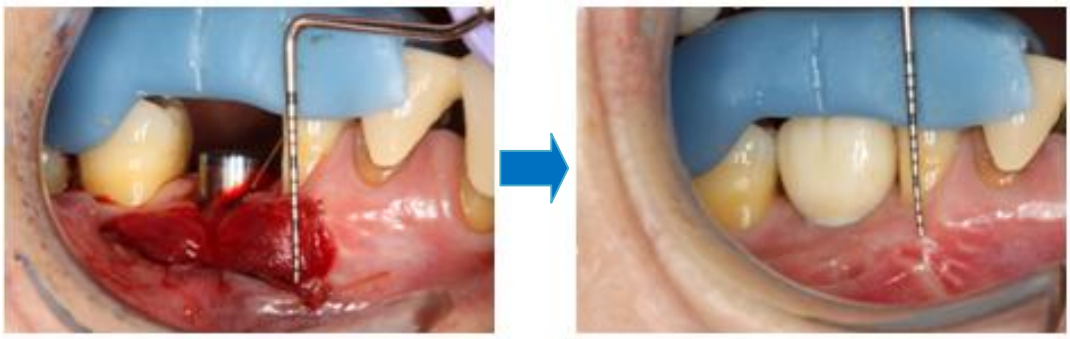




Measurement of keratinized mucosa at 3 month follow- up – Apically positioned flap with Mucograft







To be completed on the day of the surgery by the patient:

ID:

1. Discomfort Survey (Date _ _/ _ _ / 20_ _)

The purpose of this study is to evaluate the level discomfort that can occur during and after gingival augmentation procedures. The information provided will be kept confidential and will not be released to third parties.

Thank you in advance for your collaboration!

Please fill in the following survey encircling the conditions that apply to your case:

1. Discomfort level during the surgery: (please circle one of the following on the scale)

No discomfort Worst possible discomfort

0 1 2 3 4 5 6 7 8 9 10

To be completed by the patient at the follow up visit: 7-10 days

ID:

1. Pain and Discomfort Survey **(Date _ _/ _ _ / 20_ _)**

The purpose of this study is to evaluate the level discomfort that can occur during and after gingival augmentation procedures. The information provided will be kept confidential and will not be released to third parties.

Thank you in advance for your collaboration!

1. **Pain assessment:** (please circle one of the following on the scale)

No pain Worst possible pain

0 1 2 3 4 5 6 7 8 9 10

2. Swelling: (please circle one of the following on the scale)

No Very noticeable

Swelling Swelling

0 1 2 3 4 5 6 7 8 9 10

3. Bleeding: (please circle one of the following on the scale)

No Bleeding

Bleeding could not be stopped

0 1 2 3 4 5 6 7 8 9 10

To be completed by the patient:

ID:

Interval period:

In each box, please record the number of pills of the prescribed pain killer taken.

	Day 0	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9	Day 10
1am											
2am											
3am											
4am											
5am											
6am											
7am											
8am											
9am											
10am											
11am											
Noon											
1pm											
2pm											
3pm											
4pm											
5pm											
6pm											
7pm											

8pm											
9pm											
10pm											
11pm											
Midnight											
Total											

The purpose of this study is to evaluate the quantity of pain killer medication taken after gingival augmentation procedures.

The information provided will be kept confidential and will not be released to third parties.

Thank you in advance for your co-operation!

Esthetic Evaluation ***

Survey should be performed:
-On implant fixtures restored with permanent prosthesis
-At 6month after the surgical procedure
-By experienced independent clinicians (2 faculty members of department of Periodontology)

Name:

Date of surgery:

Date of survey (Photograph taken, if photograph used):

LOCATION (please check the appropriate box):

▢ Maxillary Left ▢ Maxillary Anterior ▢ Maxillary Right

▢ Mandibular Left ▢ Mandibular Anterior ▢ Mandibular Right

0 = least favorable, 10 = most favorable

- **Level of the gingival margin**: Location of gingival margin with respect to the restored prostheses.

  0 1 2 3 4 5 6 7 8 9 10
  Please circle one

- **Marginal tissue contour**: Shape and adaptation of the keratinized gingiva around the final prostheses.

  0 1 2 3 4 5 6 7 8 9 10
  Please circle one

- **Soft tissue texture**: Overall texture and physical characteristics of the gained keratinized gingival.

  0 1 2 3 4 5 6 7 8 9 10
  Please circle one

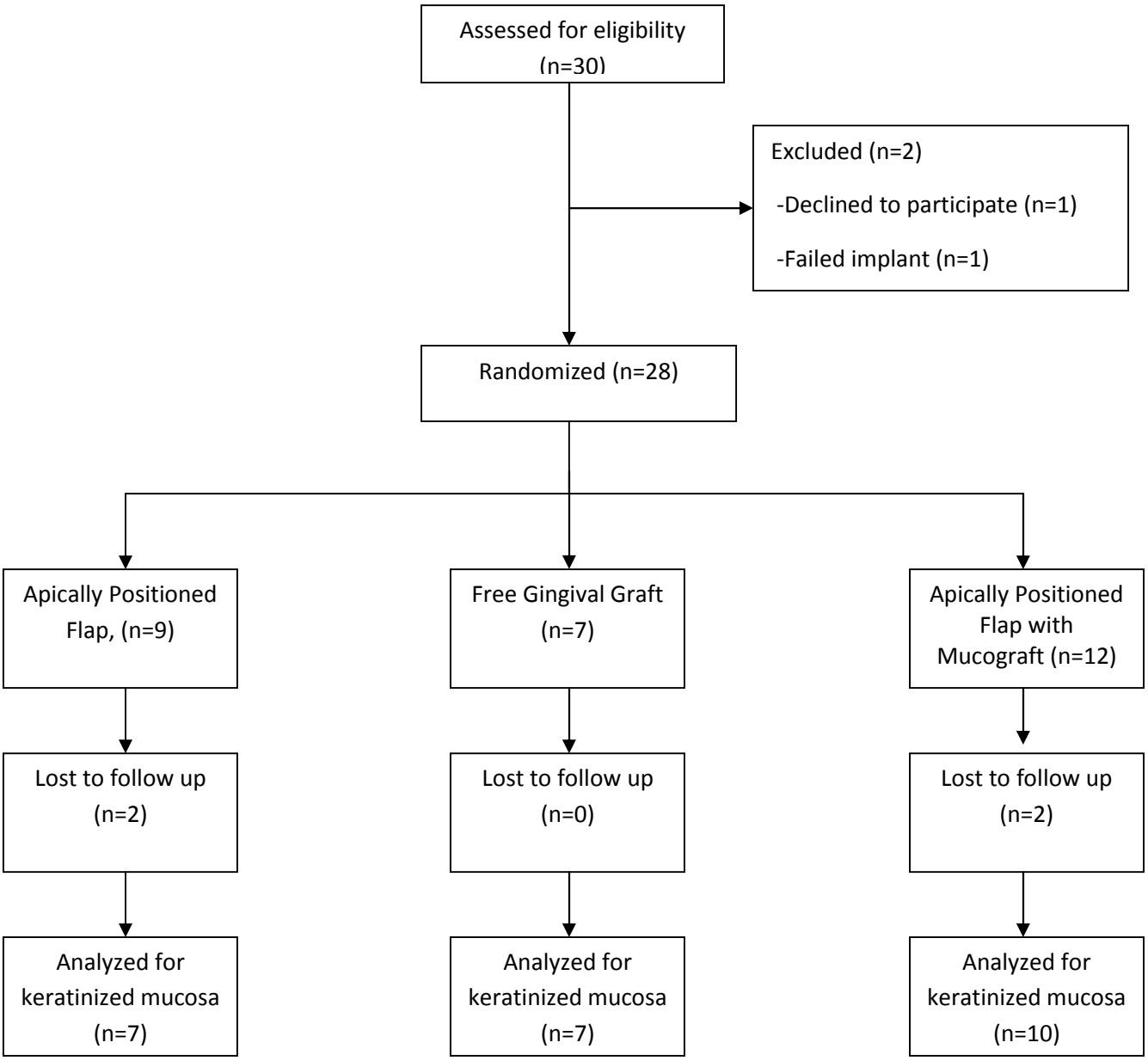
- **Mucogingival junction alignment**: Location and shape of the mucogingival junction on the gained keratinized gingival area, and its blending into adjacent areas.

  0 1 2 3 4 5 6 7 8 9 10
  Please circle one

- **Gingival color**: Blending of color with surrounding areas.

  0 1 2 3 4 5 6 7 8 9 10
  Please circle one

Subjects Flow Chart



Biography

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