Evaluation of the Effects of Two Dressing Materials: Absorbable Collagen Wound Dressings (HeliCOTE®) and Oxidized Regenerated Cellulose (Surgicel®) on Wound Healing at the Palatal Donor Site after Free Gingival Graft Surgery: A Pilot Study.

A Thesis

Presented to the Faculty of Tufts University School of Dental Medicine

in Partial Fulfillment of the Requirements for the Degree of

Master of Science in Dental Research

by

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August 2015

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ABSTRACT

Aim & Hypothesis: The aim of the study was to evaluate the effects of Absorbable Collagen Wound Dressings (HeliCOTE®) and Oxidized Regenerated Cellulose (Surgicel®) on palatal wound healing rate and pain after free gingival graft surgery using clinical measurements and Visual Analogue Scales (VAS). The hypothesis was faster wound healing would be observed among palatal wounds covered by Absorbable Collagen Wound Dressings (HeliCOTE®) compared with the Oxidized Regenerated Cellulose (Surgicel®) group. Also, compared to Oxidized Regenerated Cellulose (Surgicel®), patients would have less pain when palatal wounds are covered by Absorbable Collagen Wound Dressings (HeliCOTE®).

Materials & Methods: 20 patients required FGG surgery were randomly assigned to one of the two experimental groups, the HeliCOTE® group or the Surgicel® group. Dressing materials were applied to the palatal wounds and stabilized by suture. Post surgical photographs were exposed at the day of surgery and post-surgical day 7 and 14 for evaluation of wound healing and the surface area of the remaining wound was calculated by photo-digital planimetry using computer software. Visual Analogue Scales (VAS) was collected at post-surgical day 3 and 7 for pain assessment. Adverse events including post-surgical bleeding, infection and necrosis of the tissue were examined and recorded at post- surgical 7 and 14 days. Following the assessment, comparison between the experimental groups on surface area change of the remaining wound, VAS pain scores and other variables were analyzed.

Results: The mean reaming wound area decreased over time for the HeliCOTE® group, however in the Surgicel® group, the mean surface area of the remaining wound increased during first post-surgical week and follow by decreased during second week post-surgically. The mean reduction in remaining wound area was statistically significant greater in the HeliCOTEl® group

compared to the Surgicek® group during the first post-surgical week (*p= 0.028*). The HeliCOTE® group also demonstrated greater reduction of mean remaining wound surface during the second post-surgical week and the overall two-week post surgical period when compared to the Surgicel® group but the difference between two groups was not statistically significant (*p= 0.153; p= 0.951*.) The mean VAS pain scores showed no statistically significant difference between two experimental groups except one of the question from post-surgical day 3.

Conclusions: The result of the present prospective clinical trial showed Absorbable Collagen Wound Dressings (HeliCOTE®) enhanced palatal wound healing when compared with the Oxidized Regenerated Cellulose (Surgicel®) group during the first post-operative week. When the time point reached to the second week the healing rate between two experimental groups became comparable. Patients experienced similar post-operative discomfort when evaluating pain assessment by VAS pain scores. Both dressings did not place patients at a higher risk of infection or other complication, and both dressings were beneficial in achieving hemostasis. Future studies might involve larger sample size, control group without dressing material and histological assessment to further understand the mechanism of action of the dressing materials.

DEDICATION

Firstly, I would like to express my sincere gratitude to my advisor Dr. Karimbux for his motivation, immense knowledge, and continuous support. His guidance lead me towards the right direction and helped me overcame the obstacles. I could not have imagined having a better advisor and mentor for my master study.

I would like to sincerely thank the outstanding committee members: Dr. Steffesen, Dr. Shapurian and Dr. Pagni for their precious suggestions and time throughout this project. It was my pleasure working with them and the project would not have finished without their efforts.

Last but not least, I would like to thank my family: my parents, my husband and my sister for the immeasurable support.

ACKNOWLEDGMENTS

I would like to thank Dr. Britta Magnuson, Mr. Jacob Silberstein and Miss Cassandra O'Connell, as their work contributed significantly for the IRB approval of the project.

My sincere thanks also goes to Dr. Chun-Tee Lee from Harvard School of Dental Medicine, who spent his precious time in the data processing. I would like to compliment Prof. Yung-Nien Sun and Mr. Chan-Pang Kuok from National Cheng Kung University, Tainan, Taiwan for giving me great advice regarding professional knowledge of computer science and digital image analysis.

Special recognition goes to my faculty and co-residents in the TUSDM Department of Periodontology, who helped me recruiting patients and supported me throughout the whole period of clinical data collection.

This study would not have been completed without their participation, and I sincerely appreciate each and every one of the contributors.

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TUSDM – Tufts University School of Dental Medicine

ACWD- Absorbable Collagen Wound Dressings

ORC - Oxidized Regenerated Cellulose

MCH - Microfibrillar Collagen Hemostat

VAS- Visual Analogue Scale

Evaluation of the Effects of Two Dressing Materials: Absorbable Collagen Wound Dressings (HeliCOTE®) and Oxidized Regenerated Cellulose (Surgicel®) on Wound Healing at the Palatal Donor Site after Free Gingival Graft Surgery: A Pilot Study.

Introduction

Mucogingival Surgery

Mucogingival deformities are defined as a departure from the normal dimension and morphology of, and/or the interrelationship between gingiva and alveolar mucosa; these abnormalities may be associated with a deformity of the underlying alveolar bone [1]. In 1972, *Lang and Löe* [2] examined the relationship between gingival width and inflammation, and concluded that 2 mm of keratinized gingiva and 1 mm of attached gingiva is considered enough in order to maintain gingival health. On the other hand, other studies demonstrated that gingival health could be maintained even with more than 2 mm of keratinized tissue and that there is lack of association between the width of attached gingiva and the development of recession[3-5]. In 1984, *Hall* [6] analyzed the contributing factors of gingival recession and divided them into two groups. The first group included predisposing factors, which were inadequate attached gingiva, high frenum attachment, malpositioned teeth and osseous dehiscence. The second group embraced precipitating factors, which included vigorous tooth brushing, laceration, recurrent inflammation and other iatrogenic factors. *Hall* concluded that the combination of predisposing and precipitating factors contribute to the pathogenesis of gingival recession.

Evidence shows that with minimal amount of keratinized and attached gingiva, periodontal health can be maintained [7, 8]. However, due to the unpredictability of patient habits or other iatrogenic factors [9-12], periodontal plastic surgery may still be required for natural teeth or dental implants in some circumstances when risk factors are present.

Soft Tissue Grafting in Periodontology

Free gingival grafts (FGG) and subepithelial connective tissue grafts (SCT) have been found to be the most commonly grafts used in periodontal plastic surgeries to correct mucogingival defects in morphology, position and/or amount of soft tissue and underlying bone support for teeth and dental implants [13]. The free gingival graft was first described by *Bjorn* in 1963 [14]. *Sullivan* and *Atkins* in 1968 then explored the feasibility and healing of the free gingival graft [15, 15-17]. The predictability and long-term stability of the surgical outcome for free gingival graft has been well established [15, 18-22], however, this surgical procedure requires autogenous graft harvesting. Harvesting the donor graft from the palate can cause longer chair time, higher possibility of healing morbidity, and intra- and/or postoperative discomfort, which can lower patient acceptance [23].

Postoperative management of donor site

The surgical technique of free gingival grafting requires harvesting of soft tissue from the palatal donor site and transplantation to the intraoral recipient site. Post-surgical complications and atypical healing processes have been described in the literature [24-28]. Postoperative pain, swelling, excessive hemorrhage, infection and graft shrinkage are the most common complications [24], additionally, bone exposure, recurrent herpetic lesions, mucoceles [25] and arteriovenous shunts have been reported [26]. In order to reduce the morbidity events associated with mucogingival surgery, there are several ways to manage the donor site after soft tissue harvesting. Use of analgesics has become a standard of care after surgery, and adjuncts such as surgical dressings, collagen matrices, different suturing methods and plastic palatal protective stents can also be used to improve patient comfort and reduce bleeding of

donor sites [29-31]. In a split mouth prospective randomized clinical trial, *Alec Yen et al.* [31]in 2007 showed that platelet concentration may accelerate wound healing and regeneration of soft tissue, however, there were no statistical differences in terms of the occurrences of complications or post-operative pain.

Hemostatic agents

Various hemostatic agents have been employed to manage post-operative bleeding at the donor site. Currently the interest is focused upon absorbable synthetic collagen, absorbable gelatin sponge, and oxidized regenerated cellulose. Side effects of application the used of hemostatic agents include foreign body reactions or retarded healing of the wound [32-34].

Surgicel®, a resorbable oxidized cellulose material, is widely applied for periodontal or oral surgery. Oxidized cellulose was first used by *Frantz* [35] in 1944 and is now well known and useful for it's handling friendly, biocompatibility and bactericidal properties [36].

It has been demonstrated that Surgicel Absorbable Hemostat® is bactericidal against broad range of gram-positive and gram- negative organisms including antibiotic resistant bacteria (MRSA, VRE, PRSP and MRSE) [37] When applied in periodontal surgery, *Pollack and Bouwsma* [38] found that the bactericidal activity of Surgicel® is non-specific and effective against several periodontal pathogens. Seven to ten days after application of the Surgicel®, the material degraded and released carboxylic acid moieties, which lower the pH. The low pH of the carboxylic acid helped local hemostatic action and then activated the platelets to form a temporary plug [39]. The low pH of carboxylic acid groups also created an acidic environment in which most of the bacteria were unable to survive [40].

Petersen et al. [41] observed two absorbable hemostatic agents: gelatin sponge (Spongostan®) and oxidized regenerated cellulose (Surgicel®) by packing the hemostatic agent into the extraction sockets and then comparing bleeding, swelling and healing after tooth extraction. The two materials did not differ in the bleeding or swelling and no complications were encountered. Neither of the two materials impaired closure of the wound, however healing was slightly delayed in the gelatin group.

Rossment et al. [42] examined oxidized regenerated cellulose and absorbable gelatin sponge in management on the bleeding of palatal donor sites, and found significant benefits in achieving hemostasis when applying hemostatic agents on palatal donor sites of free gingival grafts compared to pressure only.

Absorbable synthetic collagen can be applied to a bleeding surface in a shredded microfibrillar form (Avitene® etc.) or as a sponge (Collastat®, HeliCOTE® etc.) *Stein* in 1985 first introduced the use of Collastat® sponges in periodontal mucogingival surgery and this material proved to be a highly useful adjunct to control of hemostasis in palatal donor sites [43].

Absorbable synthetic collagen wound dressings for dental surgery (eg. Collaplug®, HeliCOTE®) has a sponge- like structure and are soft, white, pliable, nonfriable and coherent, which is easy to control when applied. These products are fabricated from bovine collagen obtained from deep flexor tendons. Greater than 90% of the volume of the absorbable collagen wound dressing consists of open pores and the dressing is able to hold excessive fluid. Clinical indication includes wound protection and control of bleeding [44].

The absorbable synthetic collagen wound dressing acts in two main ways. Firstly, it serves as a mechanical obstruction to bleeding and, secondly, the biological properties of

collagen affect the coagulation process. Upon contacting blood, collagen can cause aggregation of platelets with subsequent binding to collagen fibrils. Platelet degranulation then occurs, with the released coagulation factors and plasma factors enabling formation of fibrin [45]. The sponge structure of the absorbable collagen dressing strengthens the blood clot by providing a three-dimensional scaffold [43, 44].

Shanmugam et al. [46] compared the effects of the collagen-based dressing material CollaCote® and with non-eugenol pack (Coe Pak® TM) as control on the healing of palatal wounds after free gingival graft harvesting. Various subjective and objective parameters were examined, including pain, color and consistency of the healing sites, and histology. The results demonstrated that the subjective and objective parameters showed significant improvement and histologically there was evidence of greater collagen formation in the collagen dressing group when compared to control group.

From the results of the previous studies, both absorbable synthetic collagen and oxidized regenerated cellulose demonstrate benefit in achieving hemostasis. Moreover the synthetic collagen dressing showed the potential for acceleration of the early healing phase.

Aim and Hypothesis

Specific Aims

- The first goal of the study was to evaluate the effects of Absorbable Collagen Wound Dressings (HeliCOTE®) and Oxidized Regenerated Cellulose (Surgicel®) on palatal wound healing rate after free gingival graft surgery using clinical measurements.
- The second objective of the study was to evaluate the effect of Absorbable Collagen Wound Dressings (HeliCOTE®) and Oxidized Regenerated Cellulose (Surgicel®) on pain after harvesting free gingival grafts from palatal donor sites utilizing a Visual Analogue Scale.

Hypothesis

- Faster wound healing would be observed among palatal wounds covered by Absorbable Collagen Wound Dressings (HeliCOTE®) compared with the Oxidized Regenerated Cellulose (Surgicel®) group.
- Compared to Oxidized Regenerated Cellulose (Surgicel®), patients would have less pain when palatal wounds are covered by Absorbable Collagen Wound Dressings (HeliCOTE®).

Research Design

This prospective randomized clinical pilot trial examining two groups was conducted at the Tufts School of Dental Medicine (TUSDM), Department of Periodontology between February 2015 and July 2015. Prior to the study, the protocol was submitted and approved by the Institutional Review Board and the study was conducted based on the protocol.

Twenty-one patients requiring free gingival graft procedures were screened by one of the investigators (T.C.S.) The selection of the participants was based on the following inclusion criteria: (1) Patient had at least one site of natural dentition or dental implants that needed to be treated by free gingival graft surgery for one of the following indications including progressive recession, planned prosthodontics, mucogingival deformity, or lack of keratinized gingiva (2) Patients had no systemic disease contraindicated to dental surgery (3) Patient had no dermal or autoimmune disease. Subject who matched one of the following exclusion criteria was not eligible for the study and was precluded. Patients were excluded from the study if: (1) They were a smoker (2) Patient had any known disease that interferes with periodontal surgery (3) Patient had dermal or autoimmune disease (4) Patient had previous adverse reaction to the products (or similar products) used in this study (5) Pregnant women (self-reported) (6) Patient had infection at the palatal site. The purpose and nature of the study were explained to the patient and an informed consent was obtained from patient who agreed to participate in this study prior any procedure of the study.

Oral hygiene instructions and non-surgical therapy were performed prior to the surgery to achieve periodontal health if necessary. No surgery was performed until patient reached an FMPS (full mouth-plaque scores, *O'Leary* et al. 1972) ≤ 20% and an FMBS (full-mouth bleeding scores, *Ainamo & Bay* 1975) ≤ 20%. Prior to the surgery, subjects receiving free

gingival graft were randomly assigned to the test group, in which the palatal wound site was covered by Absorbable Collagen Wound Dressings (HeliCOTE®); and to the control group, in which Oxidized Regenerated Cellulose dressing (Surgicel®) was applied.

Materials and Methods

Study materials

One of the two different post-surgical dressing materials was applied at random to the palatal donor site after free gingival graft harvesting. An Absorbable Collagen Wound Dressings (HeliCOTE ®; Integra LifeSciences Cop, NJ, USA) made from collagen obtained from bovine deep flexor (Achilles) tendon was applied to the test group. Oxidized Regenerated Cellulose dressing (Surgicel ®; Johnson & Johnson Medical, TX, USA), which is a non-collagen based absorbable hemostatic agent, was applied to the control group.

Surgical procedure

The surgeries were performed by senior residents in the postgraduate periodontal specialty program and supervised by faculty members. Free gingival graft surgical procedure for either natural teeth or dental implants were performed based on similar protocols. Autogenous free gingival grafts were obtained from the superficial layer of the palate, the recipient bed was prepared and the free gingival graft was stabilized to the recipient bed. The whole procedure of free gingival graft surgery was performed based on the principles of the classical approach described by *Sullivan and Atkins* [15,16]. After the free gingival graft was harvested from the palatal donor site, a dressing material, Absorbable Collagen Wound Dressings (HeliCOTE ®) or Oxidized Regenerated Cellulose dressing (Surgicel ®) was trimmed to the size of the wound and applied to the palatal donor site by the surgeon, following the manufacturer's instructions. The surgeon paid attention to avoid the wound margin covered by the selected dressing material for the accuracy of wound size measurement on the clinical photographs. X-

cross sutures, 5-0 Vicryl®, inserted 2 mm away from the wound margin, were placed to stabilize the dressing material after hemostasis was achieved.

After finishing the surgery, post surgical information was collected on a standardized case report form and clinical photographs were taken with and standard-sized visual scale (aluminum foil, 5.5mmx 2mm) by the same investigator (T.C.S.) for further analysis. The information collected on the case report forms included the date and the name of the surgeon performing the surgery, area of the surgical procedure, and details of the surgical procedure such as type and quantity of anesthesia, operation time, complications during the procedure, and type and quantity of the medications prescribed. Clinical measurement of the size of the harvested graft was also documented. The width and the length of the graft were measured by a standard periodontal probe to the nearest millimeter. The standard probe was also placed next the harvested graft, perpendicular to the horizon, to measure the thinnest and thickest point of the graft and then the thickness of the graft was categorized into one of the three categories based on the range of thickness [16].

Postsurgical care

Immediately following the procedure, an ice pack was administered while post-operative instructions were given (both written and verbal). A standardized selection of analgesics was offered to the patient: Ibuprofen or Tylenol was prescribed along with one kind of narcotic for all of the patients. All patients were instructed to rinse with 0.12% chlorhexidine gluconate mouthrinse twice per day for 2 weeks. Moreover, specific instructions on how to complete the surveys were explained to each participant. To ensure subjects understood how to complete

the VAS accurately each patient completed a *primer* VAS under the supervision of the investigator (T.C.S.). (Appendix B Figure 2)

Patients were seen after 7 (± 1 day) and 14 days (± 1 day) for postoperative follow-up. Sutures were removed from the palatal donor site after 1 week and from the recipient sites after 2 weeks. Clinical photographs include standard-sized visual scale (aluminum foil, 5.5 mm x 2 mm) were exposed by one investigator (T.C.S) during each follow-up visit.

Data analysis

A. Aim 1: Difference in remaining wound area (mm^2)

Clinical photographs of the surgical sites were collected on the day of the surgery and on 7 and 14 days after the surgical procedure. A digital SLR camera (Nikon D7000) with macro lens (Nikon Micro-NIKKOR 105mm f/2.8 Lens) and ring flash (Sigma EM-140 DG Macro Ring Flash for Nikon AF) was utilized to capture clinical photographs. Photographs were taken by one investigator (T.C.S.) and the settings of the camera were always consistent. The investigator paid specific attention capturing the wound area in a perpendicular way by utilizing intra-oral mirrors. The standard-sized visual scale (aluminum foil, 5.5 mm x 2 mm) was placed next to the wound as the reference for the wound size measurements and analysis. Two blinded examiners (T.S. and C.T.L.) traced both the wound remaining area and the reference visual scale independently by a computer software program (Microsoft Paint) using a mouse based on the following criteria: examiners

traced the wound edges by identifying the fibrinous tissue [54], fibrin, slough, scab, granulation tissue or necrosis tissue which were not covered by epithelium and appeared to have different color, tissue texture match when comparing with the adjacent unwound area. Immature re-epithelized area around the wound edges, tissue undergoing the process of remodeling and usually with the appearance of erythematous due to inflammation, was not considered as wound remaining area. Ten de-identified photographs obtained from palatal donor sites of other free gingival graft surgical procedures were selected for calibration prior the actual tracing. Both examiners traced each of the photographs twice for calculation of the intra-examiner reliability. The results of the calibration demonstrated excellent (almost perfect) intra (ICC score= 0.999 and 0.999) and inter (ICC score= 0.998 and 0.991) examiner reliability. The tracing of experimental clinical photographs obtained form the study objects started after the calibration.

Sixty clinical photos in total, taken from 20 patients at three different time points, were used for the final analysis. In order to calculate intra- examiner reliability during wound tracing, fifteen photos were randomly selected from the pool of 60 study photos using software R (version 3.1.2.) By adding fifteen repeated photos, 75 photos were traced by the same two blinded independent examiners (T.S. and C.T.L.) following a randomized sequence generated by using the software R (version 3.1.2,) and utilizing Microsoft Paint computer software and mouse.

After tracing by two independent examiners (T.S. and C.T.L,) the total 150 traced pictures were imported into a software program (ImageJ 1.49v; The National Institutes of Health, USA http://imagej.nih.gov/ij/) and wound areas were calculated by computerized planimetry. This semi-automatic software analyzed a digital photograph and measured the number of pixels of the two selected areas traced by the examiner, including both the area of the reference scale and the wound remaining area. By comparing the number of pixels of the area of reference visual scale to the actual surface area of the standard-sized visual scale (aluminum foil, 5.5 mm x 2 mm,) the surface area (mm^2) of the wound remaining area was then calculated for each clinical photograph.

B. Aim 2: Pain assessment

Subjects were instructed to fill out a questionnaire, which was designed to evaluate pain and postoperative complications during the first two weeks of healing. The questionnaire included two parts. For patients who could not read English, the questionnaire was translated to their respective native language for better understanding. Part one utilized a Visual Analogue Scale (VAS) adapted from a previously validated patient- reported oral mucositis symptom scale (PROMS) [47] to evaluate the postoperative discomfort. The patient was asked to fill out Part One on the 3rd and 7th day after the surgery. Part Two included the information of quantity and type of analgesic medication taken by subjects through self-report on the 7th day after the surgery. Information about post-operative complications, including tissue necrosis, ecchymosis, swelling

or infection, or additional medication was documented in the case report form by clinical investigators when the patient was examined on day 7 and day 14 after the surgery.

Statistical Analysis

Subjects were randomly assigned in a 1:1 ratio to Group 1 (HeliCOTE ®) or Group 2 (Surgicel ®) using a computer-generated randomization scheme (www.random.org). Fifteen photos were randomly selected from the sixty study photos using software R (version 3.1.2. R Foundation for Statistical Computing. Vienna, Austria.) for calculation of intra-examiner reliability. By adding fifteen repeated photos, a total of 75 photos were traced by two independent examiners with a randomized sequence generated by using the software R (version 3.1.2.) to calculate the surface area of the remaining wound (mm^2.)

The intraclass correlation coefficient for calibration and final data was calculated using a two-way mixed-effects model to compare inter- and intra- examiner reliability for wound tracing with Image J computer software (ImageJ 1.49v; The National Institutes of Health, USA http://imagej.nih.gov/ij/).

Descriptive statistics such as age and surface area were compared between groups using an *independent sample t-test*. Gender was compared between groups by the *Fisher's exact test* and graft thickness was compared between groups by the *Chi-square test*. The mean surface area (mm^2) of the remaining wound at the day of surgery, at 7 and at 14 days after the surgery of each subject was calculated. The percentage of the remaining wound compared to the original wound size at 7 and at 14 days after surgery was also calculated.

The mean difference of the remaining wound surface area (mm^2) at different time points was compared between groups using an *independent sample t-test*. Complications were compared between groups by the *Fisher's exact test*. VAS scores were compared between groups. The *independent sample T test* was used for normally distributed data (Day 3;Q 4,5,7,8,9,), and the *Mann-Wittney U test* was used for the data that was not normally

distributed. The percentage of the remaining wound area on day 7 and day 14 were compared between groups by the *Mann-Whitney Test*.

Pearson correlation was used to test the correlation between mean differences of the surface area (mm^2) of the remaining wound and the size of the wound at different time points. *Spearman correlation* was used to analyze the correlation between mean differences of the surface area (mm^2) of the remaining wound the graft thickness at different time points within different groups.

Statistical analyses were performed using SPSS (version 22, IBM Corp. Armonk, NY.) Any *p-value* less than 0.05 were considered statistically significant.

Results

Inter- and intra- examiner reliability

Inter-examiner reliability when tracing the remaining wound area and also the reference standard-sized visual scale was calculated by intraclass correlation coefficient (ICC) using a two-way mixed-effects model. The kappa coefficient, defined by *Landis* and *Koch* in 1977 was used for the result interpretation. The inter-examiner ICC scores calculated for remaining wound area (mm^2) tracings were 0.987 and 0.986 for pixel of the reference scale. Both scores indicated excellent (almost perfect) inter-examiner reliability. The individual ICC for pixel of reference scale tracing of 0.986 indicated excellent (almost perfect) inter-examiner reliability. The intra- examiner reliability was also calculated, and the individual ICC score was 0.996 for Examiner One and 0.999 for Examiner Two indicating excellent (almost perfect) reproducibility when the examiners performed second tracings on the same photograph.

Changes in wound surface areas

Twenty patients in total were randomly distributed to the test and control groups before the surgical treatment. There were no statistically significant differences when comparing age, gender, wound size (mm^2), and thickness of the graft (3 category) between the two groups [16]. (APPEDEIX A, Table 1)

The measurement of the remaining wound area of the ACWD (HeliCOTE®) group continuously decreased through out the whole observation period except for two wounds, which experienced an increase in size during the first week only, followed by a reduction in size during the second week. The mean of the remaining wound areas was 121.31 ± 43.52

mm^2 on the day of surgery, 97.60-± 44.12 mm^2 on postoperative day 7 and 29.80 ± 26.18 mm^2 on postoperative day 14.

In comparison, the remaining wound areas of the ORC (Surgicel®) group increased among 5 out of 10 patients on day 7 after the surgery. The mean surface area change demonstrated a tendency toward an increase in size during the first week of healing followed by a decrease during the second week. The mean of the remaining wound area was 105.92 ± 46.96 mm^2 on the day of surgery, 110.87± 55.87 mm^2 on postoperative day 7 and 42.16 ± 39.80 mm^2 on postoperative day 14. (APPEDEIX A, Table 3)

Difference of remaining wound surface area (mm^2) was our primary outcome parameter for comparing the progression of healing between the two experimental groups. When analyzing changes within treatment groups, the mean differences in remaining wound surface area between the day of surgery and day 7 post-surgeries for the ACWD (HeliCOTE®) group was 23.71 ± 28.49 mm^2 and for the ORC (Surgicel®) group was -4.95 ± 24.86 mm^2; 67.80 ± 34.36 mm^2 (HeliCOTE® group) and 68.71 ± 30.87 mm^2 (Surgicel® group) between day 7 and 14 post -surgery; the difference were 91.51 ± 43.67 mm^2 (HeliCOTE® group) and 63.76 ±39.44 mm^2 (Surgicel® group) between the day of surgery and day 14 post-surgery. When comparing the healing between the two experimental groups, the reduction in mean remaining wound surface area was statistically significant greater in the HeliCOTE® group compared to the Surgicel® group during the first post-surgical week (*p= 0.028*). ACWD (HeliCOTE®) group also demonstrated greater mean remaining wound surface area changes during the second post-surgical week and the overall two-week post surgical period when compared to the Surgicel® group but the difference between two groups was not statistically significant (*p= 0.153; p= 0.951.*) (APPEDEIX A, Table 2)

When comparing the % of healing at different time points, at postoperative day 7, HeliCOTE® group had a mean 78 ± 24 % of remaining wound area. The % of healing was statistically significant (*p= 0.041*) when compared to the Surgicel® group which had a mean 101 ± 29% of remaining wound area on post- surgical day 7. However, there was no statistically significant difference when comparing the % of remaining wound area on postoperative day 14 between two groups (*p=0.198*). (APPEDEIX A, Table 4)

For the HeliCOTE® group, correlation between the wound size and the wound surface area change from the day of the surgery to day 7 post- surgery was not statistically significant (*Pearson correlation, r= 0. 306 p= 0.390*). There was a positive correlation between wound size and the difference of the wound surface area between post-surgical day 7 and 14 (*Pearson correlation, r= 0.788 p= 0.007*), and also positive correlation between wound size and the difference of wound surface area between the day of the surgery and post- surgical day 14 (*Pearson correlation, r= 0.820 p= 0.004*) in the HeliCOTE® group. For the Surgicel® group a positive correlation was found between wound size and the difference of the wound surface area between post- surgical day 7 and 14 (*Pearson correlation, r= 0.854 p= 0.002.*) For both experimental groups, there was no correlation between the thickness of the graft and the difference of the wound surface area between different time points.

Post-surgical complication

There were no cases of infection or necrosis at the donor site for either experimental group. Two out of 10 patients experienced bleeding in the HeliCOTE® group and 3 out of 10

patients in the Surgicel® group. The difference in number of complications between the two groups was not statistically significant (*p=1.000*). (APPEDEIX A, Table 1)

Post- surgical pain assessment

The mean VAS pain score for each of the question presented in APPEDEIX A, Table 5. Only the score for Day 3 VAS Q7 demonstrated a significant difference between 2 groups. Patients in the HeliCOTE® group reported more pain when brushing their teeth during the first 3 days after surgery compared to patients in Surgicel® group. The mean VAS pain scores for the other 21 questions showed no statistically significant differences between two experimental groups.

Discussion

The present study aimed to assess healing of the palatal donor site and pain after free gingival graft harvesting procedure comparing two dressing material, HeliCOTE® and Surgicel®. A prospective, randomized clinical trial was designed to assess differences between two groups during the first two postoperative weeks.

Post- operative management of the palatal donor site had been widely discussed in literature. Suture, wire ligature to keep periodontal dressing (Coe-Pak®) in place, Hawley appliance [48], palatal protection stent [42, 49, 50] different dressing material including microfibrillar collagen hemostat (MCH) [30], absorbable collagen wound dressing [43], absorbable gelatin sponge, oxidized regenerated cellulose [42], and growth factor for example platelet concentration[31], medicinal plant extract (MPE) [50] are different methods that have been described. Several dressing material have been described in the literature to enable successfully protecting the palatal wound without additional coverage of a palatal stent [30, 43]. In the current study, two most commonly using dressing materials in the TUSDM periodontal clinic, oxidized regenerated cellulose (Surgicel®) and absorbable collagen wound dressing HeliCOTE® were applied for postoperative care of the donor site and secured with sutures. The wound protection ability of the two dressing materials and the impact on wound healing were assessed without a palatal stent as a bias factor.

The primary outcome in the study was wound healing. The surface area change of the surgical wound were monitored and compared between the two groups. During the first week period, HeliCOTE® group demonstrated statistically significant faster healing than the

Surgicel® group. Statistically significant differences were found for both surface area change and percentage of the remaining wound area, the HeliCOTE® group has greater reduction in wound surface area from the day of surgery to post-surgical day 7 and less percentage of the remaining wound area at post surgical day 7. From post surgical day 7 to day 14, two experimental groups showed similar rates of healing. For an overall two week post-surgical period, the HeliCOTE® group had more mean surface area reduction (91.51 ± 43.67 mm^2) and less mean percentage of remaining wound area (23 ± 23 %) when compared to the surgicel® group (63.76 ±39.44 mm^2; 36 ± 29 %) but the difference between two groups was not statistically significant. *Franoush*[48] concluded that the palatal donor site healed by secondary intention and time for completion of healing was about 2 to 4 weeks depending on the width and the thickness of the graft being removed in a review study discussed about different technique for palatal wound protection. *Thoma et al*. [51]concluded collagen matrix (CM) improved early wound healing when comparing collagen matrix covered palatal wound to palatal wound healing left by secondary intention. In their study, the wound area was 3 mm deep and 28.26 mm^2 in size after surgery, and the mean surface area change during the first 8 days post-surgery was 15.56 ± 2.4 mm^2 for the CM group and 14.66 ± 2.8 mm^2 for wound healed by secondary intention. By the second week, both groups achieved complete epithelialization. In this current study, for an overall two weeks post-surgical period, both the HeliCOTE® and the Surgicel® groups showed comparable results when compared the wound surface area changes to previous study by *Thoma et al*. [51] However, during the first week post- surgical period, only HeliCOTE® group showed similar healing tendency and superior result compared to previous data[51]. Within the Surgicel® group, wound area experienced an increase in size during the first week of healing.

Studies had demonstrated both HeliCOTE® and Surgicel® are useful for hemostasis control in palatal donor sites [42, 43]. However, their mechanisms of actions are still not completely understood. Literature[41-43] described the Absorbable Synthetic Collagen Wound dressing (HeliCOTE®) serves as a mechanical obstruction to bleeding and the biological property of the collagen affects the coagulation process. Oxidized Regenerated Cellulose (Surgicel®) has a physical hemostatic effect, it swells in contact with blood, adheres to the wound edges and vessels forming an artificial coagulum rather than an alteration of the physiologic clotting mechanism. In current investigation, 2 out of 10 patients from HiliCOTE® group and 3 out of 10 patients from Surgicel® group experienced post operative bleeding during the fist 3 post operative days, the difference between two experimental groups was not statistically significant. Compared to result from *Rossmann*'s study [42], none of the 9 patients in the gelatin sponge group developed any adverse event, while 4 out of 9 donor sites in the ORC group and gauze-only group, respectively, developed adverse events, the incident of postoperative bleeding in the current study is lower. In this study, none of the patients experienced infection or necrosis of the tissue on palatal donor sites during two weeks healing period. This finding coincident with other reports.

The resorption rate of Surgicel® and HeliCOTE® had been described in the literature. According to previous reports, both Surgicel® and HeliCOTE® resorbed in 7-10 days [38, 44]. In current study, none of the patients reported that the dressing material dislodged during the two weeks postoperative period, and only 5 out of 20 patients experienced bleeding. During clinical examination at postoperative day 7, none of the wounds showed evidence of the

remaining dressing material. The resorption rates of the materials in current study were similar to previously reported data [38, 44].

Side effects of application of hemostatic agents had been reported in the literature, including foreign body reaction or retarded healing of the wound. [32-34] In our study the majority of patients in the Surgicel® groups experienced increasing or no change in wound size during the first week period. Matthew et al in 1993 [34] examined tissue responded to oxidized regenerated cellulose (Surgicel®) in tooth extraction sockets clinically and histologically when compared to extraction sockets filled with blood clots in an animal model. The results showed that oxidized regenerated cellulose delayed wound healing in the early phase of healing (1-4 weeks) giving rise to foreign body reactions. At a microscopic level, Matthew described after one week, there was still implanted Surgicel® material present surrounded by macrophages and fibroblasts in the connective tissue. Also *Ibarrola et al.* in 1985 [32], when observing osseous reaction to implanted Surgicel® from an animal study, showed Surgicel® markedly slowed the rate of repair and caused inflammation leading to an inhibitory effect of proper bone healing. In the present study, the clinical observation of an increase in wound size within the Surgicel® group during first week post-operative period showed that the hemostatic agent oxidized regenerated cellulous (Surgicel®) might retard the soft tissue healing in the initial phase of healing. The wound-healing rate of the Surgicel® group then increased and showed no statistically difference when compared to the HeliCOTE® group during the period of postoperative second week. Future study with histological analysis might help us to further understand the mechanism of action and also how soft tissue healing was impacted by Surgicel® at a microscopic level.

HeliCOTE®, an absorbable synthetic collagen wound dressing is fabricated from bovine collagen obtained from deep flexor tendons and is used to protect the wound and control bleeding [44]. *Stein et al.* [43] introduced collagen sponge (Collastat®) as a topical hemostatic agent in mucogingival surgery and explained the sponge form of an absorbable collagen enhances platelets aggregation, entrapping plasma coagulation factors and subsequent clot formation. The histologic evaluations of human palatal tissue, 42 days after the surgery demonstrated evidence of more collagen formation in the collagen-dressing group when compared to control group according to *Shanmugam et al.* [46]. Although the reason why absorbable collagen wound dressing enhanced the quality of wound healing is still unclear, the clinical findings in our study revealed faster palatal wound healing in HeliCOTE® group when compared to wound dressed by oxidized regenerated cellulose (Surgicel®.) The difference was significant and evident in the first week of healing. For the two-week post-surgical period, the mean wound surface area also decreased more in the HeliCOTE® group but the overall result was not statistically significant.

In this study, the VAS pain scores for 11 questions were examined at postoperative day 3. The mean VAS pain scores of the HeliCOTE® group were higher than the Surgicel® group but only the score for question 7 had a statistically significant difference between the two experimental groups. The mean VAS pain scores for 11 questions at postoperative day 7 were higher in the HeliCOTE® group when compared to the Surgicel® group but the difference was not statistically significant. Pain is an inherently subjective multifactorial experience and has proven to be more complex to assess, evaluate, and manage [55]. In this study, the VAS pain scores reported by different patients had a large variety within the same group. Some patients

reported no pain while others indicated they experienced “the worst pain ever” after the surgery. The sample size in the current study is small and therefore larger samples might be able to give more reliable results.

Several methods have been used to study wound healing and epithelialization in the previous literature, such as direct clinical inspection, examination of clinical photographs, utilization of hydrogen peroxide solution and staining agent [30, 46, 49-52]. Direct inspection alone is more subjective and often difficult to achieve accurate and reproducible wound measurements. Application of hydrogen peroxide or staining agent, although as objective methods to assess areas of complete epithelialization, was associated with adverse events [53]. Previously, *Thoma et al.* [51]described the use of photographs in evaluation of human palatal wounds and the images were imported into computer software for wound area measurement. In current study, examination of clinical photographs was chosen as methodology to assess wound healing. Additionally, to enhance accuracy of wound size measurement, the concept of photo- digital planimetry was employed.

To the author’s knowledge, reliability of the intraoral wound area measurement by photo-digital planimetry has seldom been reported in the dental field. In our study, tracing of the remaining wound area was completed by two independent examiners based on the modified criteria from *Kahnberg*’s study. *Kahnberg& Thilander* [54] in 1982 studied excisional wound healing of palate in rat model, after leaving the bone exposed. Histological examination revealed that epithelialization progressed from the wound borders and wound surface covered by fibrin/ fibrinous layer reduced when epithelial cell migration proceeded. Inflammatory

cells were still present in the connective tissue after epithelialization with erythematous appearance. In present investigation examiners traced the wound edges by identifying the fibrinous tissue, fibrin, slough, scab, granulation tissue or necrosis tissue which were not covered by epithelium and appeared to have different color, tissue texture match when comparing with the adjacent unwounded area. Immature re-epithelized area around the wound edges, tissue undergoing remodeling process with the appearance of erythema due to inflammation, was not considered as wound remaining area. The result of the tracing for the independent examiners showed excellent reliability and reproducibility, confirming the validity of the methodology and the accuracy of study findings.

There were some limitations and several issues can be taken into consideration in the future study for the same filed of interest. First, there was no histological assessment to further support clinical findings in this study. Second, the surgery was performed as a routine treatment and the size of the graft was determined by the need of the recipient site rather than having a standardized dimension for the study purpose. In the past, several methods had been described for obtaining standardized size graft from palatal donor site however sometimes additional tissue might be removed for study purpose [49, 51]. Although the size and the depth of the wound were statistically equally distributed in our study, the methodology of the current study could be further improved for better standardization when compared to the relevant studies. Another drawback was the timeframe of the follow up period was relatively short and further data might be obtained by increasing the postoperative follow-up time until the wound is completely healed. Future studies might also recruit larger sample sizes and an additional control group without dressing material to better confirms the findings from the current study.

Conclusion

In conclusion, the result of the present prospective clinical trial showed Absorbable Collagen Wound Dressings (HeliCOTE®) enhanced the palatal wound healing when compared with the Oxidized Regenerated Cellulose (Surgicel®) group during the first post-operative week. At the second week follow up, the healing rate between the test and control groups became comparable. Patient experienced similar post-operative discomfort when evaluating pain assessment by VAS pain scores. Both dressings did not place patients at a higher risk of infection or other complication, and both dressings proved to be beneficial in achieving hemostasis.

This study supports the use of Absorbable Collagen Wound Dressings (HeliCOTE®) and/ or Oxidized Regenerated Cellulose (Surgicel®) dressing material for dressing the donor site wound when performing free gingival grafts procedure. Future studies might involve larger sample size, control group without dressing material and histological assessment to further understand the mechanism of action of these dressing materials.

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APPENDICES

Appendix A: Tables

Appendix B: Figures

Appendix A: Tables

Table 1. Demographic information

	HeliCOTE®	Surgicel®	p-value
Number of patient	10	10	
Age (y/o)	61.10 ± 12.27	60.70 ± 13.39	.945
Gender (M/F)	2/8	6/4	.170
Size of the wound (mm^2)	121.30 ± 43.52	105.91 ± 46.96	.457
Thickness of the graft			
0.75-1.25mm	0	1	
1.25- 1.75mm	7	5	.478
>1.75mm	3	4	
Number of complication (Bleeding)	2	3	1.000

Table 2. Primary outcome: difference of remaining wound area (mm^2)

	Day S- Day 7	Day 7-Day14	Day S- Day14
HeliCOTE®	23.71 ± 28.49	67.80 ± 34.36	91.51 ± 43.67
Surgicel®	-4.95 ± 24.86	68.71 ± 30.87	63.76 ±39.44
p-value	.028*	.951	.153

* $p < 0.05$

Table 3. Remaining wound area (mm^2)

	Day of surgery	Day7	Day14
HeliCOTE®	121.31 ± 43.52	97.60 ± 44.12	29.80 ± 26.18
Surgicel®	105.92 ± 46.96	110.87 ± 55.87	42.16 ± 39.80

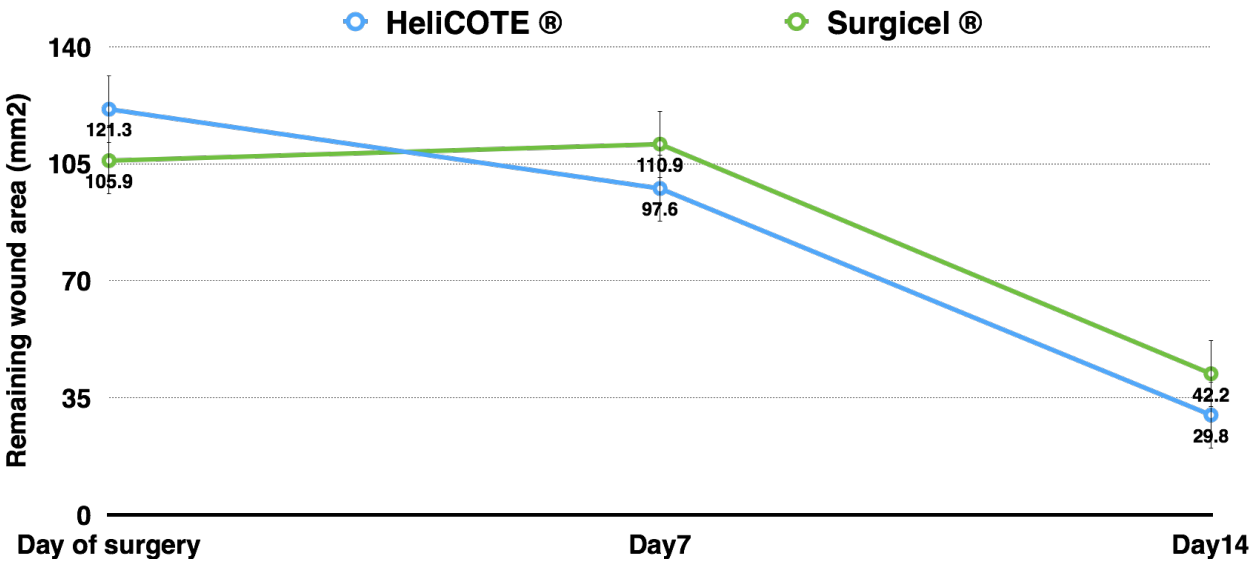

Table 4. % of the remaining wound area

	Day7	Day14
HeliCOTE®	78 ± 24	23 ± 23
Surgicel®	101 ± 29	36 ± 29
p-value	.041*	.198

* $p < 0.05$

Table 5. Secondary outcome: VAS Score

Day3	Q1	Q2	Q3	Q4	Q5	Q6	Q7	Q8	Q9	Q10	Q11
HeliCOTE®	3.9 ± 3.3	5.0 ± 3.4	3.5 ± 3.2	3.9 ± 2.7	4.8 ± 3.4	3.1 ± 2.9	4.3 ± 2.2	4.1 ± 2.6	3.8 ± 2.4	3.8 ± 2.8	2.7 ± 2.7
Surgicel®	3.1 ± 3.5	2.6 ± 3.3	1.4 ± 1.7	2.1 ± 2.3	2.2 ± 2.0	1.9 ± 3.3	1.1 ± 1.5	2.0 ± 2.2	2.2 ± 2.3	2.1 ± 2.9	2.1 ± 2.9
p-value	.684	.075	.089	.139	.071	.237	.002*	.072	.089	.123	.481
Day7	**Q1**	**Q2**	**Q3**	**Q4**	**Q5**	**Q6**	**Q7**	**Q8**	**Q9**	**Q10**	**Q11**
HeliCOTE®	2.8 ± 3.5	3.2 ± 3.9	3.0 ± 3.9	3.6 ± 3.6	3.3 ± 3.9	2.7 ± 3.3	3.3 ± 3.3	2.9 ± 3.7	2.9 ± 3.5	3.3 ± 3.8	2.4 ± 3.8
Surgicel®	2.1 ± 2.6	2.2 ± 2.9	1.8 ± 2.2	2.0 ± 2.7	1.9 ± 2.5	1.7 ± 3.0	1.8 ± 2.2	1.9 ± 2.7	2.0 ± 2.5	1.7 ± 2.3	1.2 ± 2.5
p-value	.739	.796	.684	.247	.549	.400	.113	.684	.796	.353	.481

* $p < 0.05$

Appendix B: Figures

Figure 1. VAS Questionnaire Part I& Part II

Appendix I

This questionnaire helps you to evaluate some post-surgical conditions that you have experienced during the healing period. Please complete this questionnaire at Day 3 and Day 7 after the surgery. All of the conditions relate to intraoral symptoms. Please help us to identify the severity of the condition by marking on the lines below the question. You can use a pen or pencil to draw a vertical line on the scale.

Part I

1. Pain experienced within the whole mouth:

_____

no pain *worst pain possible*

2. Pain experienced (on the roof of the mouth) during speech:

_____

no pain *worst pain possible*

3. Pain experienced (on the roof of the mouth) during swallowing:

_____

no pain *worst pain possible*

4. Pain experienced when eating soft foods (mashed potatoes, pudding, etc.):

_____

no pain *worst pain possible*

5. Pain experienced while drinking warm beverages:

_____

no pain *worst pain possible*

7. Pain experienced while brushing teeth:

no pain _____ *worst pain possible*

8. Pain experienced in the morning when you wake up:

no pain _____ *worst pain possible*

9. Pain experienced throughout the day:

no pain _____ *worst pain possible*

10. Pain experienced at night before bed:

no pain _____ *worst pain possible*

11. Difficulty sleeping because of palatal pain:

no problem _____ *unable to fall asleep*

Part II

1. Did you experience bleeding (i.e., "bright red" color in saliva)?

YES NO

2. Did you take the prescribed pain medication?

YES NO

3. If YES, how often and how many pills did you take?_____

_____

4. Other than the medication prescribed by your doctor, did you get medication over the counter?

YES NO

5. If YES, what was the name of the medication?

_____

6. How often and how many pills did you take?

Figure 2. *Primer* VAS

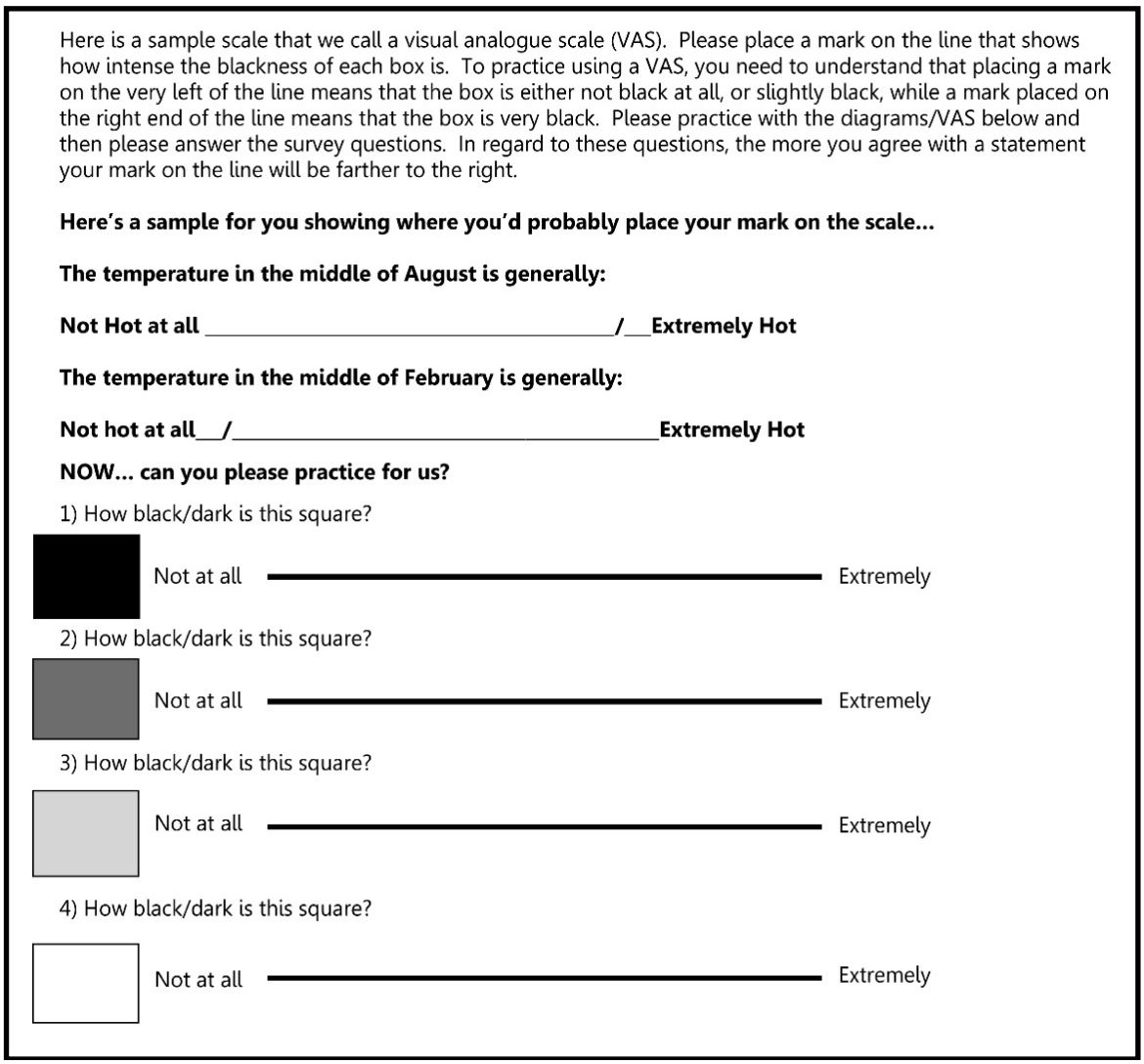

Figure 3. Overview of the clinical photographs of the surgical wound: HeliCOTE® group

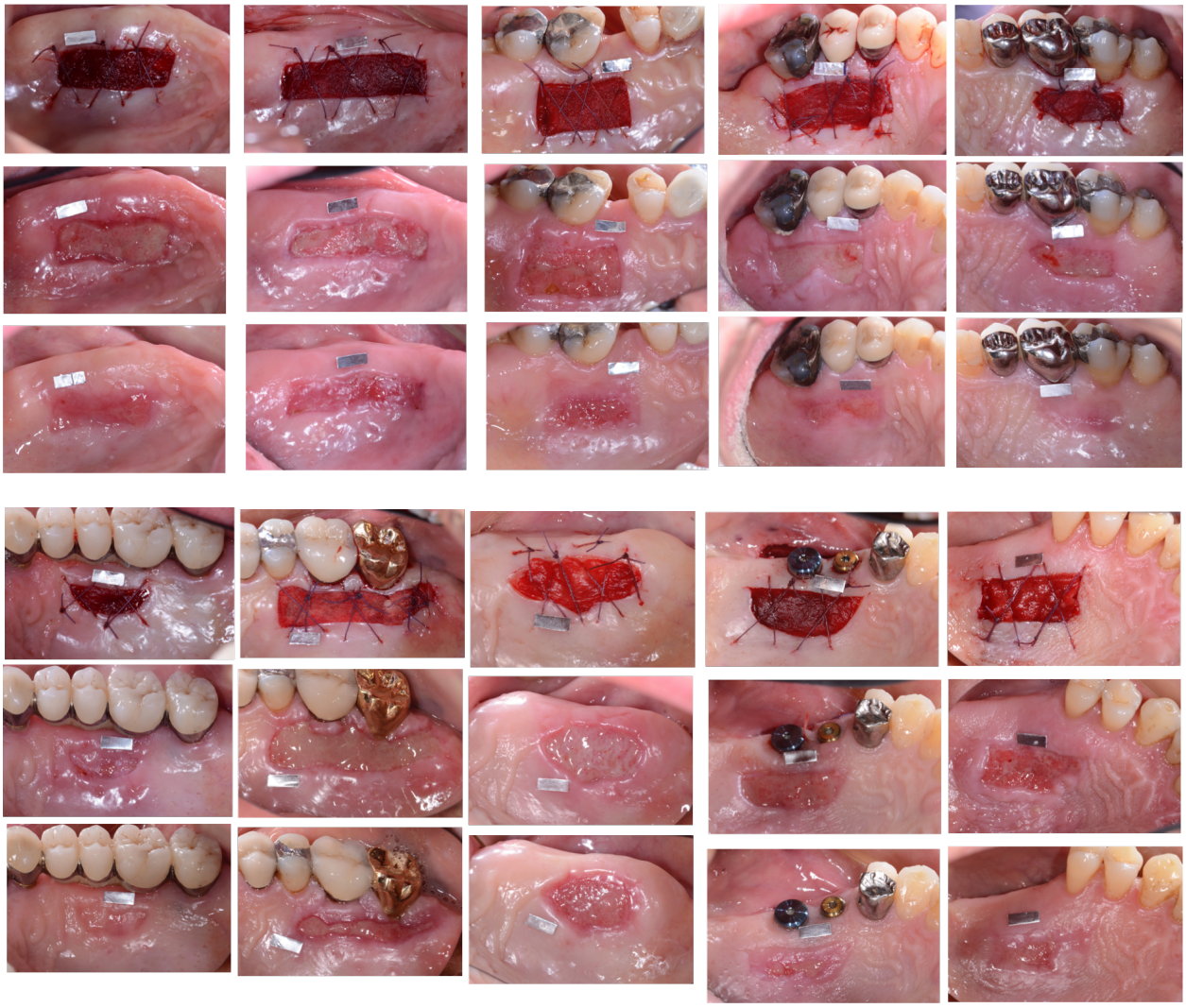

Figure 4. Overview of the clinical photographs of the surgical wound: Surgicel® group

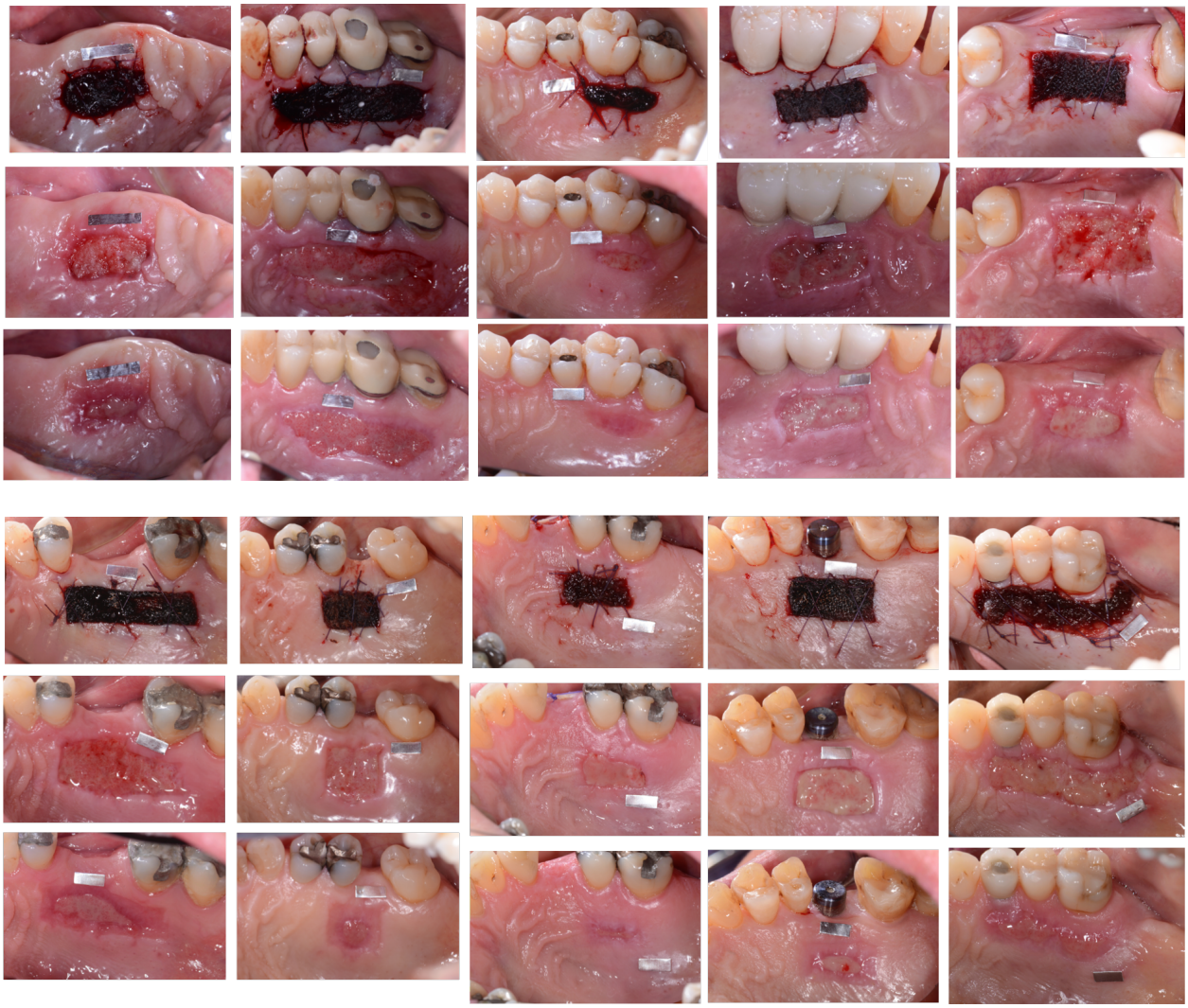

Figure 5. Overview of wound measurement on soft ware *ImageJ*