

**Patient Outcomes and Gingival Blood Flow using Laser Doppler Flowmetry
Following the use of episil® on Free Gingival Graft Donor Sites**

NCT03350724

Version Date: 10/06/2015

Protocol Title

Patient Outcomes and Gingival Blood Flow using Laser Doppler Flowmetry
Following the use of episil® on Free Gingival Graft Donor Sites

Principal Investigator

Jennifer D. James M.B.A., D.M.D.

Co-Investigators

Robin Weltman D.D.S., M.S.

Sridhar V.K. Eswaran B.D.S, M.S., M.S.D.

Population

The population will consist of 40 subjects, 18 years of age and over, females (non-pregnant or non-breast feeding) and males, with a general health status of ASA 1 or 2, and patients of record at UTHealth School of Dentistry in Houston, Texas requiring the gingival augmentation procedure, the free gingival graft. [Augmentation of gingival tissues may be considered an elective procedure. Elective surgical procedures are not routinely performed on pregnant and lactating women. Such procedures can be performed after childbirth and/or completion of lactation.] A total of 60 subjects will be consented to account for some subject's unwillingness or inability to continue in the study and non-compliance with study procedures and appointments.

Number of Sites

This will be a single site study at UTHealth School of Dentistry in Houston, Texas.

Study Duration

The duration of the study will be two years.

Subject Duration

The duration per subject will be three weeks.

Background, Aim, & Hypothesis

The free gingival graft (FGG) is a commonly used periodontal plastic surgery procedure for the augmentation of keratinized gingiva around teeth and implants. The FGG procedure uses a harvesting technique from the palate that leaves an open wound that heals by secondary intention, which can take 2 to 4 weeks. Mucogingival surgery has been shown to cause postoperative pain 3.5 times as much pain as osseous surgery and 6 times as much pain as soft tissue surgery². When bone is left exposed it tends to exaggerate the inadvertent postsurgical inflammatory response and increase postoperative pain. Wessel and Tatakis¹ compared patient outcomes following subepithelial connective tissue graft (SCTG) and FGG procedures and indicated that FGG was associated with a greater incidence of donor site pain compared to SCTG donor site during the early postoperative period. There were significant differences between early (3-day) and late (3-week) pain levels for FGG donor sites. Pain in the palatal donor site at 3 days postoperatively was reported by

50% of SCTG subjects and 90% of FGG subjects; this difference was statistically significant. A previous study⁴ found similar results and attributed the poorer patient outcomes following FGG to differences in donor site harvesting techniques. In an 8-week observational study using a verbal descriptor scale to assess postoperative discomfort, Del Pizzo et al.⁴ reported postoperative discomfort at palatal donor sites during the first postoperative week in 100% of subjects treated with an FGG harvesting technique and in only 50% of subjects treated with a single-incision harvesting technique. Additionally, in a study on 228 subjects using questionnaires to assess postoperative pain, Griffin et al.³ reported FGG subjects were three times more likely than SCTG subjects to develop post-surgical pain during the first postoperative week.

Improving patient outcomes is important in clinical practice. Wessel and Tatakis¹ found that even with the use of palatal stents to protect FGG donor sites, a greater incidence of donor site pain was found during the early healing period compared to SCTG. The results of their study suggested that there is opportunity to improve the postoperative protocols of commonly used soft tissue grafting procedures with more effective donor wound protection schemes.

At the present time, some type of wound covering over the palatal donor tissue site is considered a standard of post-operative care. While palatal stents are one option for protection of the palatal wound, other techniques have been utilized to aid in hemostasis and post-operative discomfort. Another technique utilizes a hemostatic agent such as Gelfoam® (absorbable gelatin compressed sponge) or Surgicel® covered with a wound dressing such as PeriAcryl®90 (a cyanoacrylate tissue adhesive).

A new wound dressing material, episil®, has shown to give rapid extended reduction of pain in cancer patients suffering from Oral Mucositis/Stomatitis (may be caused by chemotherapy or radiotherapy). The intended use of episil® is for the management of pain and relief of pain, by adhering to the mucosal surface of the mouth, soothing oral lesions of various etiologies. episil® has obtained 501(k) market clearance by the US FDA, in June 2011. episil® is an oromucosal liquid in a multi-dose container and is without active pharmaceutical ingredients and without systemic side effects demonstrated in clinical and pre-clinical studies. episil® is intended for use in the oral cavity as far as the pharynx but it is not dangerous to swallow. The oromucosal liquid transforms *in situ* to a bioadhesive oromucosal gel by uptake of small amounts of aqueous fluid. episil® contains six ingredients glycerol dioleate, soy phosphatidyl choline (lecithin), ethanol, propylene glycol, polysorbate 80 and peppermint oil which are all generally recognized as safe. No side effects have been reported. episil® contains small amounts of ethanol (alcohol), less than 100 mg per dose, which may cause a burning sensation when applied to sore mucosal surfaces. episil® contains also small amounts of propylene glycol which may cause skin irritation and peppermint oil which may cause allergic reaction. episil® should not be used on patients allergic to any of the ingredients, including peanuts, soy (soya) or peppermint oil. Currently there are no known concerns of use of episil® in human pregnancy or breast-feeding.

episil® is contained within an amber glass bottle with a manual metering pump filled with 10 mL of oral liquid. It is applied to the oral cavity by spraying 1 – 3 times. Distributed to affected areas in the mouth and within a few minutes the liquid converts to a gel and the protective film is formed. It is intended for use 2 – 3 times a day or as needed and is normally intended for continuous use for not more than 30 days.

Oral mucositis in advanced stages, similar to a FGG donor site, can be extremely painful, preventing a patient from eating and even disrupting the patient's daily functions. The aim of this study will be to compare patient-based outcomes, specifically postoperative pain, at the FGG donor sites, using an interventional parallel-group design, whereby a hemostatic gauze, Surgicel®, will be placed within all of the palatal donor sites and covered with either episil® (test) or a cyanoacrylate dressing, PeriAcryl®90 (control). The secondary aim of this study will be to compare gingival healing via measurement of the gingival blood flow at the FGG donor sites by the laser Doppler flowmetry (LDF) technique, by monitoring blood perfusion. The hypothesis for the present study is that patient-based outcomes and gingival blood flow will be more favorable for the FGG donor sites being covered by the test wound dressing material episil® compared to the control dressing.

Study Population and Design

Forty subjects will be recruited from the Periodontics and Dental Hygiene Clinic and the UTDentists Practice, located at the UTHealth School of Dentistry in Houston, Texas, where the study will be performed. Only subjects who have already formally agreed to receive a FGG as part of their overall periodontal treatment plan will be recruited. Inclusion criteria will be 18 years of age or older, patient of record at UTHealth School of Dentistry Houston Texas, signed treatment plan for a FGG, and the ability to provide research informed consent. Exclusion criteria will be any allergies to any of the ingredients in episil® including allergies to peanuts, soy or peppermint oil, smokers, pregnant or breast feeding women, and inability or unwillingness to provide informed consent. As stated above, elective surgical procedures are not routinely performed on pregnant or lactating women and are postponed until after childbirth and or completion of lactation. Exit criteria will be unwillingness or inability to continue in the study and non-compliance with study procedures and appointments. The Institutional Review Board of The University of Texas will approve the study protocol, questionnaires and informed consent form.

The study will be of an interventional parallel-group design. A computer generated randomization scheme will assign the subjects to one of the two treatment groups. All subjects will have the gingival grafting procedure, the free gingival graft, performed at sites lacking attached keratinized gingival tissues. The palatal donor site in subjects assigned to the control group will receive the hemostatic agent, Surgicel®, covered by the cyanoacrylate dressing, PeriAcryl®90. The palatal donor site in the subjects assigned to the test group will receive the hemostatic agent, Surgicel®, covered by episil®. Additionally, the subjects in the test group will be given the remainder of the bottle of episil® to take home with

instructions for self-application as needed 3 times daily for pain relief. A sufficient supply of the episil® will be provided to the test subjects for the study duration. The total duration of the study per subject will be 21 days from the day of the surgical procedure. The primary outcome of postoperative pain will be determined by the subjects recording their postoperative pain threshold using a Visual Analog Scale Form/Questionnaire and the number of analgesic pills taken each day at 1, 2, 3, 5, 7, 10, 14 and 21 days postoperatively. The Visual Analog Scale Form/Questionnaire for post-operative days 1, 2, 5 and 10 will be completed at the subject's place of residence, while the Visual Analog Scale Form/Questionnaire for days 3, 7, 14 and 21 will be completed at the subject's post-operative appointments at the dental clinic. The Visual Analog Scale Form/Questionnaire will evaluate the subjects' postoperative pain using a visual analog scale (VAS) scores from 1 to 10, with 1 indicating minimal pain and 10 indicating severe pain. If no pain is present, a score of 0 will be given. The levels of postoperative pain will be classified as none to minimum if the score is "0 to 3," moderate for "4 to 6," and severe for "7 to 10." None to minimal pain will mean little or no discomfort; moderate is any pain that bothers the subject and mildly affects normal function; and severe will be considered any pain that will not be tolerated and may even disrupt the subject's daily functions.

One of the secondary outcomes, gingival blood flow, will be determined with the laser Doppler flowmetry (LDF) technique to measure the blood perfusion of the FGG donor site and as well as the contralateral side of the palate, as a positive control, at baseline prior to surgery, immediately after harvesting of the FGG from donor site, and on post-operative days 3, 7, 14 and 21. The LDF technique is an unbiased non-invasive method of monitoring the response to periodontal therapy. It is adequate for recording changes in gingival blood flow following periodontal surgery presenting different patterns of microvascular blood flow alterations during the wound-healing period.¹⁸ The LDF technique is based on the Doppler principle. A laser beam will be emitted by an optical fibre onto the FGG donor site. The light hitting moving erythrocytes is scattered back in shifting frequency, which is the Doppler effect, and is captured by one or more optical fibers. The light signals are then converted into electric signals and the resulting photocurrent is processed to provide a recording of the blood flow.¹⁹ The term used to describe blood flow is flux – a quantity proportional to the average speed of the blood cells and their concentration. This is expressed in arbitrary perfusion units (PU), which are linearly related to flux. The PeriFlux System 5000 (Laser Doppler Blood Perfusion Monitoring and $tcpO_2/tcpCO_2$) along with the Laser Doppler Probes for the PeriFlux System 5000 will be used to perform the LDF technique.

The other secondary outcome will be the time needed to obtain a complete re-epithelialization of the FGG donor site, and will be performed by a non-invasive peroxide test.^{20, 21} This test is based on the principle that if the epithelium is discontinuous, the H_2O_2 diffuses into the connective tissue; the enzyme catalase acts on H_2O_2 to release water and oxygen: this is clinically shown by the production of bubbles on the wound. 3% H_2O_2 will be applied to the FGG donor site with a syringe and the appearance of bubbles will be recorded as a dichotomous variable (yes/no) at the post-operative days 3, 7, 14, and 21 until a subject's FGG donor site has

completely epithelialized (no bubbles observed), when they will not be measured again at future post-operative visits.

Data and Safety Monitoring

Subjects will be informed that if they experience any adverse events with the use of episil®, that they should discontinue the use of the product immediately. Depending on the severity of the adverse event the patient will be advised to either call 911 or Dr. Jennifer D. James to report the event. An Unanticipated Problems Tracking Log Form will be used to report adverse events to report them to CPHS. Any adverse event will then be immediately reported to Camurus AB, the manufacture of episil®.

An Unanticipated Problems Tracking Log Form will be used to record and report any adverse events that are unexpected, related and put the subjects or others at harm, meet the definition of Unanticipated Problems Involving Risks to Subjects or Others and will be reported to the Committee for the Protection of Human Subjects.

Surgical Procedures & Postoperative Care

As stated above, periodontal residents under the direct supervision of periodontal faculty will perform the FGG procedure in all subjects. The FGG procedure will be performed following the surgical technique of Sullivan and Atkins^{15,16} or Miller¹⁷. The donor tissue for the FGG procedure will be retrieved from either the right or left side of the palate. The FGG donor sites will be of a standardized size of 10 mm x 10 mm. As stated above, subjects in the control group will receive a hemostatic gauze, Surgicel®, covered by a cyanoacrylate dressing, PeriAcryl®90, at the donor site while the subjects in the test group will receive the same brand of hemostatic gauze, Surgicel®, but covered with episil®. Subjects in the test group will be given the remainder of the bottle of episil® with instructions for home application up to 3 times daily. Postoperative instructions will include discontinuing tooth brushing and flossing around the surgical site for the first 3 post-operative weeks. Subjects will be provided with a prescription for ibuprofen (600 mg) to take as needed for analgesia, unless contraindicated. Acetaminophen with 30mg of codeine will be prescribed to subjects unable to ingest ibuprofen.

The LDF measurements in the FGG donor site and on the contralateral side of the palate will be recorded in four sites. A clear acrylic stent will be fabricated for each subject from individual subjects' dental casts. Holes will be made in the stent in four locations on each the right and left sides of the palate. One site located centrally, one close to the mesial, one close to the distal and one close to the apical of the FGG donor site and in the same locations on the contralateral side of the palate will be measured at each of the specified postoperative appointments. The LDF measurement will be performed at baseline prior to surgery, immediately after harvesting of the FGG donor site, and on post-operative days 3, 7, 14 and 21. The LDF measurements will be performed with the tip of the fibre optic probe inserted into the holes of the individual acrylic stent.²⁰ The LDF probe will be placed at a

standardized location perpendicularly to the tissues and at a distance of 0.5 mm from the tissues and will remain motionless during the LDF measurements.

Below is a summary of the timeline of the study:

First clinic visit (Screening visit): Discussion of study and attain informed consent for participation in the study; medical history will be updated, vital signs will be taken, and an upper arch alginate impression will be taken in order to create a plastic stent, which will be worn during the laser Doppler flow measurements.

Second clinic visit (Day of Surgery): The day of surgery, the laser Doppler flowmetry readings will be performed before the surgery and then again immediately after harvesting of the FGG donor site. Subjects will receive 4 copies of the Visual Analog Scale Form/Questionnaire to be completed at their homes on days 1, 2, 5, and 10 postoperatively, which will be labeled accordingly. Subjects assigned to the test group will receive a manual-metering pump containing sufficient episil® for the duration of the study. Subjects in the test group will be instructed to use the pump up to 3 times a day. Instructions for home application of the episil® will be given orally and in writing at this appointment.

Day 1 post-operative: All subjects will complete at home the Day 1 Post-Operative Visual Analog Scale Form/Questionnaire.

Day 2 post-operative: All subjects will complete at home the Day 2 Post-Operative Visual Analog Scale Form/Questionnaire.

Day 3 post-operative (3rd clinic visit): All subjects will bring the 2 completed Visual Analog Scale Forms/Questionnaires to the day 3 post-operative clinic appointment and will complete the Day 3 Post-Operative Visual Analog Scale Form/Questionnaire during this appointment. The laser Doppler flowmetry readings will be performed on the free gingival graft donor site and on the contralateral side of the palate utilizing the fabricated stent with holes for the Doppler probe positioning for repeatability of readings. 3% H₂O₂ will be applied to the FGG donor site with a syringe and the appearance of bubbles will be recorded as a dichotomous variable.

Day 5 post-operative: All subjects will complete at home the Day 5 Post-Operative Visual Analog Scale Form/Questionnaire.

Day 7 post-operative (4th clinic visit): All subjects will bring completed Visual Analog Scale Form/Questionnaire to the day 7 post-operative appointment and will complete a Day 7 Post-Operative Visual Analog Scale Form/Questionnaire during that appointment and laser Doppler flowmetry readings will be performed on the free gingival graft donor site and on the contralateral side of the palate. 3% H₂O₂ will be applied to the FGG donor site with a syringe and the appearance of bubbles will be recorded as a dichotomous variable.

Day 10 post-operative: All subjects will complete at home the Day 10 Post-Operative Visual Analog Scale Form/Questionnaire.

Day 14 post-operative (5th clinic visit): All subjects will bring completed Visual Analog Scale Form/Questionnaire to the day 14 post-operative appointment and will complete a Day 14 Post-Operative Visual Analog Scale Form/Questionnaire and laser Doppler flowmetry readings will be performed on the free gingival graft

donor site and on the contralateral side of the palate. 3% H₂O₂ will be applied to the FGG donor site with a syringe and the appearance of bubbles will be recorded as a dichotomous variable.

Day 21 post-operative (6th clinic visit): All subjects will attend the day 21 post-operative appointment and will complete a Day 21 Post-Operative Visual Analog Scale Form/Questionnaire and laser Doppler flowmetry readings will be performed on the free gingival graft donor site and on the contralateral side of the palate. 3% H₂O₂ will be applied to the FGG donor site with a syringe and the appearance of bubbles will be recorded as a dichotomous variable.

Below is a chart of the timeline of the study:

Procedure	Visit 1 Screening	Visit 2 Day of Surgery	Visit 3 3 Day Post-op	Visit 4 7 Day Post-op	Visit 5 14 Day Post-op	Visit 6 21 Day Post-op
Medical History	√	√	√	√	√	√
Vital Signs	√	√	√	√	√	√
Complete Visual Analog Scale/ Questionnaire			√	√	√	√
Doppler Flowmetry (FGG donor site)		√	√	√	√	√
Doppler Flowmetry (Contralateral side)		√	√	√	√	√
3% H ₂ O ₂ (FGG donor site)			√	√	√	√

Statistical Analysis

Descriptive statistics will be expressed as means +/- SD and frequency distributions. Intergroup (FFG donor site using episil® and Surgicel® (test) and FGG donor site with PeriAcryl®90 and Surgicel® (control)) comparisons at 1, 2, 3, 5, 7, 10, 14 and 21 days will be analyzed by the Mann-Whitney U test. The Wilcoxon signed-rank test will be used for intragroup comparisons between 1, 2, 3, 5, 7, 10, 14 and 21 days. Differences in frequency distributions between the episil® group and the PeriAcryl®90 group will be analyzed by the Fisher exact test. Associations

between pain VAS scores and the number of analgesic pills or number of days analgesics were taken will be examined by the Pearson correlation coefficient. The significance level for rejection of the null hypothesis will be set at $\alpha = 0.05$.

All LDF recordings, which may be impaired by artifacts caused by the relative motion of the probe, will be excluded. An average of the 2-minute period of each individual recording will be calculated. Changes of blood flux values in the FGG donor site will be expressed as the difference (ΔPU) between the PU value at a specific site at a specific observation time point (PU_t) and the individual baseline value of the same site (PU_0): $\Delta\text{PU}_t - \text{PU}_0$.

Descriptive statistics will be performed using the SPSS statistical software. The ΔPU values in the FGG donor site will be analyzed using the general linear model (GLM) univariate test, after verification of the assumptions of homogeneity of variance and normality of the residual distribution. The observation time and point and the position (mesial, center, distal and apical to the FGG donor site) will be modeled as fixed factors and the patient as a random factor with the ΔPU as the dependent variable. The Dunnett two-sided *t*-test will be used, in order to evaluate the differences between baseline and subsequent time points and between different positions in the FGG donor site during the overall observational period. Data will be presented as mean SE. Statistical significance will be accepted at $p < 0.05$.

The outcome analysis of the time needed to obtain complete re-epithelialization of the FGG donor site will be made by comparing experimental groups on the frequency distribution of patients experiencing complete re-epithelization in each of the 4 post-operative visits. The Williams-adjusted log-likelihood ratio was performed, followed by *post-hoc* tests using Bonferroni-corrected Fisher's exact test (2-tailed).

Based on a sample size of $n = 40$ (20 per group), a repeated measures analysis will have 80% power to detect an effect size of $f = .57$. For a sample size of $n = 30$ (15 per group), the same analysis will have 80% power to detect an effect size of $f = .67$, while a sample size of $n = 20$ (10 per group) will have 80% power to detect an effect size of $f = .83$. All of these effect sizes are classified as large effects by Cohen's guidelines (1988), or large differences in the change in pain score between the two groups²².

Ethics

The Committee For the Protection of Human Subjects will seek IRB approval. Subjects will be required to sign the Informed Consent Form, which includes the following sections, invitation to take part in the research project, purpose, procedures, time commitment, benefits, risks and/or discomforts, alternatives, study withdrawal, in case of injury, costs, reimbursement and compensation, confidentiality, new information, and questions.

Data Handling & Record Keeping

Subjects will not be identifiable directly or through identifying information. The data will not be able to be linked to the subjects during the study. The Post-

Operative Visual Analog Scale Form / Questionnaire will be collected from the patient and stored by the principal investigator, Dr. Jennifer D. James.

References

1. Wessel JR, Tatakis DN. Patient Outcomes Following Subepithelial Connective Tissue Graft and Free Gingival Graft Procedures. *J Periodontol* 2008;79:425-430.
2. Curtis JW Jr., McLain JB, Hutchinson RA. The incidence and severity of complications and pain following periodontal surgery. *J Periodontol* 1985;56:597-601.
3. Griffin TH, Cheung WS, Zavras AI, Damoulis PD. Postoperative complications following gingival augmentation procedures. *J Periodontol* 2006;77:2070-2079.
4. Del Pizzo M, Modica F, Bethaz N, Priotto P, Romagnoli R. The connective tissue graft: A comparative clinical evaluation of wound healing at the palatal donor site. A preliminary study. *J Clin Periodontol* 2002;29:848-854.
5. Saroff SA, Chasens AI, Eisen SF, Levey SH. Free soft tissue autografts. Hemostatis and protection of the palatal donor site with a microfibrillar collagen preparation. *J Periodontol* 1982;53:425-428.
6. Bonica, J.J.: *The Management of Pain*. 1st ed, London, Henry Kimpton, 1953.
7. Seymour J, Clark D, Winslow M. Pain and Palliative Care: The Emergence of New Specialties. *Journal of Pain and Symptom Management* 2005;29:2-13.
8. Cooper SA, Wagenberg B, Eskow R, Zissu J. Double-blind evaluation of suprofen and aspirin in the treatment of periodontal pain. *Pharmacology* 1983;27(Suppl. 1): 23-30.
9. Betancourt JW, Kupp LI, Jasper SJ, Farooqi OA. Efficacy of ibuprofen-hydrocodone for the treatment of postoperative pain after periodontal surgery. *J Periodontol* 2004;75:872-876.
10. Pearlman B, Boyatzis S, Daly C, et al. The analgesic efficacy of ibuprofen in periodontal surgery: A multicentre study. *Aust Dent J* 1997;42:328-334.
11. Vaughan ME, Garnick JJ. The effect of a 0.125% chlorhexidine rinse on inflammation after periodontal surgery. *J Periodontol* 1989;60:704-708.
12. Tucker PW, Smith JR, Adams DF. A comparison of 2 analgesic regimens for the control of postoperative periodontal discomfort. *J Periodontol* 1996;67:125-129.
13. Hadjieva T, Cavallin-Stahl E, Linden M, Tiberg F. Treatment of oral mucositis pain following radiation therapy for head-and-neck cancer using a bioadhesive barrier-forming lipid solution. *Supportive Care Cancer* 2014;22:1557-1562.
14. Pettit L, Sanghera P, Glaholm J, Hartley A. The use of MuGard™, Caphosol® and Episil® in patients undergoing chemoradiotherapy for squamous cell carcinoma of the head and neck. *Journal of Radiotherapy in Practice* 2014;13:218-225.
15. Sullivan HC, Atkins JH. Free autogenous gingival grafts. 3. Utilization of grafts in the treatment of gingival recession. *Periodontics* 1968;6:152-160.

16. Sullivan HC, Atkins JH. Free autogenous gingival grafts. I. Principles of successful grafting. *Periodontics* 1968;6:121-129.
17. Miller PD Jr. Root coverage using a free soft tissue autograft following citric acid application. Part 1: Technique. *Int J Periodontics Restorative Dent* 1982; 2(1):65-70.
18. Stern MD, Lappe DL, Bowen PD. Continuous measurement of tissue blood flow by laser Doppler spectroscopy. *American Journal of Physiology* 1977; 28:1746-1751.
19. Retzepi M, Tonetti M, Donos N. Gingival blood flow changes following periodontal access flap surgery using laser Doppler flowmetry. *J Clin Periodontol* 2007; 34:437-443.
20. Silva CO, Ribeiro EdelP, Sallum AW, Tatakis DN. Free gingival grafts: graft shrinkage and donor-site healing in smokers and non-smokers. *J Periodontol* 2010;81:692-701.
21. D'Arcangelo C, De Ninis P, Paolantonio M. Clinical Comparison of Platelet Rich Fibrin and a Gelatin Sponge in the Management of Palatal Wounds Following Epithelialized Free Gingival Graft Harvest: A Randomized Clinical Trial. *J Periodontol* 2015;DOI: 10.1902/jop.2015.150198.
22. Cohen, J. The analysis of Variance. In: *Statistical Power Analysis for the Behavioral Sciences* 2nd ed. Hillsdale, NJ: Lawrence Erlbaum Associates; 1988:273-406.