# Comparative Histological Evaluation of Intra- and Extraorally De-epithelialized Connective Tissue Graft Samples Harvested from the Posterior Palate Region

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**Total word count (excluding abstract and references):** 2836

**Number of tables and figures:** 6 figures.

**Running Title:** De-epithelialization of connective tissue grafts.

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This is the author manuscript accepted for publication and has undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the Version of Record. Please cite this article as doi: 10.1902/jper.11059.

**One sentence summary:** Intraoral and extraoral de-epithelialization of connective tissue graft samples are comparably effective.


## Abstract

**Background:** Autologous connective tissue graft (CTG) is generally considered the gold standard for peri-implant soft tissue phenotype modification and root coverage therapy. The presence of epithelial remnants in CTG has been associated with complications after soft tissue augmentation surgery. However, a specific method for de-epithelization that is patently superior has not been identified yet. This study aimed to evaluate the effectiveness of two different approaches to de-epithelialize CTG samples harvested from the posterior palate.

**Materials and Methods:** Patients in need of periodontal or implant-related surgery that required harvesting a CTG from the posterior palate region were recruited. CTG samples harvested with an indirect approach were de-epithelialized using either an intraoral (IO group) or an extraoral (EO group) method. Tissue specimens were subsequently processed for histological analysis. The presence or absence of oral epithelial remnants was determined by two examiners using light microscopy.

**Results:** Twenty-four patients, who provided a total of 46 analyzable CTG samples, were enrolled in this study. Histological assessment revealed that 19 out of 22 samples in the IO group were free of epithelial remnants. In the EO group, 20 out of 24 samples did not exhibit epithelial residues. These results translate into 86.4% and 83.3% of epithelium-free samples in the IO and EO groups, respectively.

**Conclusions:** Although the intraoral method may provide several practical advantages compared to the extraoral technique, both approaches for de-epithelialization of CTG samples harvested from the posterior palate region tested in this study were comparably effective. However, none of these methods ensured complete removal of the oral epithelium in a predictable manner, which should be taken into consideration in clinical practice.


**Keywords:** clinical decision-making, phenotype, plastic surgery, oral mucosa, histology

## 1. Introduction

According to current evidence, the autogenous connective tissue graft (CTG), also known as subepithelial connective tissue graft, can be considered the "gold standard" for periodontal and peri-implant soft tissue augmentation procedures. [1-4] Possible intraoral donor regions for CTG are the anterior and posterior palate, the tuberosity, and the retromolar pad.[5] The choice of the donor site is primarily based on the clinician's preferences considering quantity, morphology, and the inherent characteristics of the CTG needed to achieve the therapeutic goal.[6] While it has been demonstrated that CTG obtained from different intraoral regions have distinct biological and structural features that should be considered in the clinical decision-making process,[7-9] the palatal region remains as the most common donor site for CTG in contemporary clinical practice, in particular the area of the hard palate delimited by the distal line angle of the canine and the mid-palatal aspect of the second molar, which is anterior to the typical location of the greater palatine foramen.[10]

Palatal CTG may be harvested through a direct approach with a classic trap door,[11, 12] a dual parallel incision method using a double-blade scalpel,[13-15] or, in a less invasive manner, with a single linear incision.[16, 17] Alternatively, the indirect harvesting approach, originally described by Bosco and colleagues in 1996 [18] and described in more detail in 2007,[19] which has been popularized in recent years by Zucchelli and coworkers,[20, 21] consists of the extraoral de-epithelialization of an autogenous free gingival graft using a scalpel blade to maximize the amount of dense lamina propria contained within the graft. Another modality is the de-epithelialization of the tissue intraorally prior to graft harvesting with the use of high-speed diamond bur under irrigation.[22, 23]

Although rare, the presence of epithelial remnants in CTG may be associated with a variety of complications after soft tissue augmentation interventions. Some authors have linked the use of CTG with external root resorption,[24, 25] gingival cul-de-sac with or without keratin discharge,[26, 27] epithelial cell discharge,[28] and cyst formation,[29-33] which may result into poor functional and esthetic outcomes. Other authors have reported the appearance of bony exostoses after the use of CTG alone,[34] CTG in conjunction with enamel matrix derivative,[35] autogenous free gingival grafts,[36, 37] and skin grafts.[38] However, it may be argued that the presence of epithelial remnants may not be an etiological factor in the onset of these conditions. Anyhow, to minimize any possible risks, de-epithelization of CTG is desirable and should be an objective in the context of periodontal and implant-related soft tissue augmentation therapy.

Among available clinical studies on this topic, there is limited information on whether a specific method to remove the epithelium from CTG is patently superior. Therefore, this study aimed to evaluate the effectiveness of two different approaches (intraoral vs. extraoral) to de-epithelialize CTG samples harvested from the posterior palate.

## 2. Materials and methods

### 2.1. Study design, ethical approval, and setting.

This study was designed and monitored according to the principles of the international Appraisal of Guidelines, Research, and Evaluation (AGREE).[39] The experimental protocol was approved by the University of Iowa Institutional Review Board (IRB) in October 2021 (HawkIRB #202109521). The clinical component of this study was conducted in the Department of Periodontics at the University of Iowa College of Dentistry and Dental Clinics between October 2021 and January 2022.

### 2.2. Eligibility Criteria and Recruitment

Adult subjects who required periodontal or implant-related surgery at the University of Iowa College of Dentistry were eligible to participate in this study. The inclusion criteria were as follows: 1) Subjects ≥18 years of age; 2) ASA status I or II; 3) Undergoing periodontal or implant-related surgical interventions that involved harvesting a CTG from the posterior palate region and would allow extending the donor region for research sample collection; 4) Adequate physical and mental health to receive routine dental treatment including the surgical procedures associated with harvesting the subepithelial connective tissue graft. The exclusion criteria were as follows: 1) Uncontrolled diabetes mellitus (HbA1c>7.0); 2) Autoimmune, infectious, inflammatory conditions, or intake of drugs known to affect the homeostasis of the oral mucosa; 3) Pregnant women or nursing mothers; 4) Severe hematological disorders (i.e., leukemia or hemophilia); 5) Currently under cancer treatment or within 18 months from completion of chemotherapy or radiation in the head and neck region; 7) History of antibiotic or immunosuppressant use within the last 3 months; 8) Current smokers. All potential participants were required to read, understand, and sign the informed consent form, which contained detailed information about the purpose of the study and potential risks.

### 2.3. Clinical Procedures

All surgical procedures were performed under local anesthesia by two different providers (E.C.Q. or G.A.O.) following a standard CTG harvesting protocol. The examiners were previously calibrated in a discussion meeting to become familiar with the study protocol and receive instructions to standardize the harvesting procedure methods. Additionally, both surgeons were together in the first five surgical interventions (10 samples). Two subsets of soft tissue samples were harvested for research purposes from the proximity the donor surgical site. Two different de-epithelization methods, one for each sample, either intraoral (IO group) or extraoral (EO group), were followed. A shallow horizontal incision was made at approximately 3mm apical from the gingival margin of the maxillary molar teeth utilizing a scalpel blade.[12] Subsequently, a parallel horizontal incision, at

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approximately 4mm apical from the first horizontal incision, was traced in conjunction with two vertical releasing incisions to delineate a rectangular area of approximately 8x4mm. An additional vertical incision was made to separate the 8x4mm sample into two equal parts, as shown in Figure 1. In the IO group, de-epithelialization was performed by removing approximately 1mm of the most superficial tissue utilizing a round diamond bur[13] mounted in a high-speed handpiece at approximately 200,000 rpm with copious sterile water irrigation. Bleeding was used as a subjective indicator of complete epithelium removal. After IO de-epithelialization, the sample was harvested in a split-thickness fashion, making an effort to maintain a uniform thickness of 2 to 3mm, as shown in Figure 2. Extraorally, the sample was completely divided into two parts utilizing a scalpel blade. Subsequently, the sample assigned to the EO group was de-epithelialized utilizing a fresh scalpel blade, as described elsewhere[19] and as illustrated in Figure 3. Samples were immediately submerged in 10% neutral buffer formalin solution for tissue fixation. All subjects received detailed verbal and written post-operative instructions according to the surgical treatment that was performed. Subjects in need for additional therapy were scheduled in the appropriate clinic at the University of Iowa College of Dentistry, and further surgical and/or restorative treatment was completed outside the study.

### 2.4. Histological Analysis

After proper fixation and dehydration in ethanol baths of increasing concentration, soft tissue biopsy samples were embedded in paraffin blocks. One individual sagittal section of 5µm was obtained from the middle portion of each sample, mounted onto a glass slide, and dried overnight. All samples were stained with hematoxylin and eosin (H&E). Standardized digital images of the whole specimen were captured using a light microscope[14] (Figure 4). Histological analyses were performed by two examiners (E.C.Q. and G.A.O.) to determine whether epithelial remnants were present or not along the entire sample. All the samples were de-identified and the examiners were not aware of which intervention the samples received until all the analyses were performed. The examiners were previously calibrated by conducting a series of five separate histological analyses in duplicate using random samples.

### 2.5. Statistical Analysis

The percentage of samples that contained epithelial remnants in each group was calculated by dividing the number of samples per group exhibiting epithelial remnants by the whole sample size and subsequently multiplying that number by 100, with the corresponding 95% confidence interval [40]. This outcome was the variable of interest in this study. Sample size was calculated considering the number of groups (n=2), with a power of 95%, using $\alpha$=0.05. A minimum of 30 autogenous subepithelial connective tissue samples, 15 in each group, was deemed necessary. Bearing in mind a deterioration of the sample upon harvesting or processing percentage of 20%, the total sample size was calculated

[13] 5801.31.016 FG Super Coarse Round Diamond, Brasseler Dental, Savannah, GA, USA

[14] Primo Star, Carl Zeiss, Oberkochen, Germany.

to be 36 in total, 18 in each group. The sample size of the study is justified based on the percentage values of epithelial remnants in CTG from a previous study.[41] Inter-rater reliability of histological analysis was assessed using inter-class correlation coefficients.[42]

## 3. Results

### 3.1. Population

Twenty-nine patients were screened and determined to be eligible. Five of them declined to participate in the study. Therefore, a total of 24 patients were recruited. Each patient contributed with two samples, one for each group, except one patient from whom four samples were obtained, two from each side of the posterior palate. This population was constituted by 10 males (41.7%) and 14 females (58.3%) between 23 and 73 years of age, with a mean age of 46.2±15.4 years. A flowchart illustrating the patients enrolled and samples analyzed is depicted in Figure 5.

### 3.2. Histological analysis

Four samples were not analyzable due to excessive deterioration upon harvesting or after histological processing. Hence, a total of 46 CTG samples, of which 22 corresponded to the IO group and 24 to the EO group, were analyzed. Perfect intra (1.00) and inter-rater reliability agreement (1.00) were demonstrated within and between examiners for all the samples. Histological analyses in the IO group revealed that 19 out of 22 samples were free of remnant epithelium. In the EO group, no epithelial remnants were observed in 20 out of the 24 samples. These results translate into 86.4±35.1% (95%CI: 71.7 to 100) and 83.3±38.1% (95% CI: 68.1 to 98.6) of epithelium-free samples in the IO and EO groups, respectively. Conversely, epithelial remnants were identified in 12.6% and 16.7% of the IO and EO samples, respectively. Images of all histological samples analyzed are displayed in Figure 6.

Two interesting patterns were observed in the samples that presented epithelial residues in both groups. While in the IO group epithelial remnants were short but present across the superficial aspect of the three samples that had epithelial remnants (#11, 13, and 18 in Figure 6), in all but one (#8 in Figure 6) of the four samples in the EO group a small portion of epithelium remnants were located only in the corner of the specimens (#6, 18, and 20 in Figure 6). Notably, no signs of thermal damage were observed on the surface of the samples de-epithelialized with the extraoral method.

## 4. Discussion

To the best of our knowledge, this is the largest human histological study to date aimed at evaluating the effectiveness of two different approaches (i.e., intraoral vs. extraoral) for the de-epithelialization of CTG samples harvested from the posterior palate region. Histological analyses revealed that the percentage of samples presenting epithelial remnants was similar between groups. In the IO group, 86.4±35.1% (95%CI: 71.7 to 100) of the CTG samples were free of epithelium versus 83.3±38.1% (95%

CI: 68.1to 98.6) in the EO group. In another similar study, de Marques de Mattos et al. observed no statistically significant differences regarding the presence of epithelium residues in CTG samples de-epithelialized either intra- or extraorally (n=10 per group). However, it is noteworthy that less epithelial rests were observed in the IO group (20%) compared with the EO group (40%).[43] The dissimilarities between studies could be explained by the dimensions of the CTG samples and the anatomical references that were used to perform the de-epithelization procedure in the EO group.

Different findings have been reported in other publications. Azar and collaborators found epithelial remnants in all CTG samples (n=5) analyzed in their study, these samples were de-epithelialized extraorally with a scalpel. The median fraction of total area corresponding to epithelial remnants was 6.01% (range 3.23% to 12.46%).[44] Similarly, Sebaoun and coworkers reported the presence of epithelial remnants in all nine CTG specimens analyzed in their study, which were also de-epithelialized extraorally. Epithelial rests occupied between 4.57% to 29.12% of the total superficial area of the samples.[45] In a study by Bakhishov and colleagues observed epithelial tissue remnants in the superficial region of all CTG samples (n=17) analyzed, which were obtained after intraoral de-epithelialization using a bur.[22] In the present study, epithelial remnants were identified in only 12.64% and 16.67% of 22 IO and 24 EO samples, respectively. These discrepancies could be explained by technical differences in the approach employed to obtain the CTG samples, intraoral location (i.e., anterior versus posterior palate), or the histological section analyzed (i.e., marginal vs. central portion), among other methodological variables.

Although no differences between groups were observed in terms of the percentage of CTG samples that contained epithelial remnants, the intraoral de-epithelialization method tested in this study was slightly superior (86.4% vs. 83.3%) and presents several potential advantages compared to the extraoral counterpart. The intraoral method may be particularly useful to remove the epithelium from CTG samples obtained from regions with an irregular, non-flat morphology such as the anterior palate, tuberosity, or retromolar region. Uniformly and efficiently de-epithelializing a CTG presenting an irregular morphology extraorally can be difficult because the sample is free (non-attached to underlying tissue), and the efficient use of a scalpel is technically challenging. For the same reasons, intraoral de-epithelialization is generally more time-efficient compared to the extraoral approach. A cost-effectiveness argument could also be made in favor of the intraoral approach as a diamond bur may be utilized multiple times, while the use of a single fresh blade, that needs to be discarded after the procedure, is generally recommended for extraoral de-epithelialization. Patients presenting thin palatal soft tissue thickness may also benefit from the intraoral de-epithelialization approach, as it allows maximization of the preservation of the connective tissue by avoiding excessive removal of

lamina propria. However, a potential limitation of intraoral epithelialization is accessibility in patients with limited mouth opening or with parapharyngeal reflex.

Different histological studies have shown interindividual variations in the dimensions, collagen cross-linking and maturation, amount of lamina propria, and proportion of fibrous and glandular tissue of CTG harvested from different regions of the palate.[7, 41, 46] A subjective factor that was taken into consideration in the IO group was the presence of bleeding upon de-epithelization with a round diamond bur. This visual cue theoretically indicates that the epithelium, which does not contain blood vessels, has been completely removed and the lamina propria is exposed. However, clinicians must be aware that this could render false positives as complete elimination of the epithelial tissue (bleeding points) may have been achieved in some areas, causing profuse bleeding that spreads over the surface of the sample, while epithelial remnants are still present, as shown in samples 11, 13 and 18 (Figure 6). To overcome this issue, a reduction of at least 1mm measured with a periodontal probe has been proposed. This recommendation is based on the thickness of the oral epithelium in the palatal region reported by Soehren and colleagues, which ranged from 111µm to 619µm with a mean value of 364µm.[47]

Interestingly, two distinct patterns of epithelial rests were observed in this study. Epithelial remnants were primarily located in the corner region of IO specimens, while in the EO group the epithelium was reduced in height but present across the superficial aspect. These findings are likely due to the difficulty to remove the epithelium around the boundaries of free CTG samples when using a scalpel blade in the EO group and insufficient vertical reduction with the use of a diamond bur in the IO group, probably because of overconfidence in the bleeding sign. Therefore, clinicians should pay attention to meticulously remove the epithelium on the peripheral aspect of CTG samples when following the extraoral approach and consistently remove at least 1 mm of superficial tissue when using a bur intraorally, regardless of the presence of bleeding.

This study has several limitations. First, only two de-epithelialization methods were evaluated. Second, all CTG samples were obtained from the posterior palate. While this was done intentionally for standardization purposes, this does not allow to extrapolate our findings to other donor sites (i.e., anterior palate, tuberosity region, or retromolar pad). Third, no additional local phenotypic (e.g., total palatal tissue thickness) or histomorphometric characteristics (proportion of lamina propria and submucosa) were assessed, mainly because this was outside of the primary scope of this study. Future investigations should further evaluate the efficacy of different and novel intra- and extraoral methods, such as Er,Cr:YSG,[48] ER:YAG,[49] or CO2[50] lasers, for de-epithelization of CTG harvested from different intraoral donor regions, and evaluate the differential effect that these methods have on patient

reported outcomes (e.g., level of self-reported discomfort during the healing period) to identify reliable while minimally invasive CTG harvesting strategies.[51]

## 5. Conclusions

Based on the findings of this study, it can be concluded that, although the intraoral method may provide several practical advantages compared to the extraoral technique, both approaches for de-epithelialization of CTG samples harvested from the posterior palate region tested in this study were comparably effective. However, none of these methods ensured complete removal of the oral epithelium in a predictable manner, which should be taken into consideration in clinical practice.

Source of Funding: No financial support or sponsorship was received.

Disclaimers: The authors have no conflicts of interest to report pertaining to the conduction of this study.

Acknowledgements: The authors would like to thank Ms. Sara Miller, research manager at the Iowa Institute for Oral Health Research, University of Iowa College of Dentistry for her efforts and support during the conduction of the study.

## References


1. Barootchi S, Tavelli L, Zucchelli G, Giannobile WV, Wang HL. Gingival phenotype modification therapies on natural teeth: A network meta-analysis. *J Periodontol* 2020;91:1386-1399.

2. Chambrone L, Botelho J, Machado V, Mascarenhas P, Mendes JJ, Avila-Ortiz G. Does the subepithelial connective tissue graft in conjunction with a coronally advanced flap remain as the gold standard therapy for the treatment of single gingival recession defects? A systematic review and network meta-analysis. *J Periodontol* 2022;93:1336-1352.

3. Chambrone L, Chambrone D, Pustiglioni FE, Chambrone LA, Lima LA. Can subepithelial connective tissue grafts be considered the gold standard procedure in the treatment of Miller Class I and II recession-type defects? *J Dent* 2008;36:659-671.

4. Tavelli L, Barootchi S, Avila-Ortiz G, Urban IA, Giannobile WV, Wang HL. Peri-implant soft tissue phenotype modification and its impact on peri-implant health: A systematic review and network meta-analysis. *J Periodontol* 2021;92:21-44.

5. Chambrone L, Avila-Ortiz G. Chapter 4: The Grafts. In: Chambrone L, Avila-Ortiz G, eds. *TISSUES: Critical Issues in Periodontal and Implant-Related Plastic and Reconstructive Surgery*. Chicago: Quintessence Publishing, 2022.

6. Zuhr O, Bäumer D, Hürzeler M. The addition of soft tissue replacement grafts in plastic periodontal and implant surgery: critical elements in design and execution. *J Clin Periodontol* 2014;41 Suppl 15:S123-142.

7. Dellavia C, Ricci G, Pettinari L, Allievi C, Grizzi F, Gagliano N. Human palatal and tuberosity mucosa as donor sites for ridge augmentation. *Int J Periodontics Restorative Dent* 2014;34:179-186.

8. Gamborena I, Blatz M. *Evolution: Contemporary Protocols for Anterior Single-Tooth Implants*. Chicago: Quintessence Publishing Co.; 2014: 440.

9. Sanz-Martin I, Rojo E, Maldonado E, Stroppa G, Nart J, Sanz M. Structural and histological differences between connective tissue grafts harvested from the lateral palatal mucosa or from the tuberosity area. *Clin Oral Investig* 2019;23:957-964.

10. Tavelli L, Barootchi S, Ravida A, Oh TJ, Wang HL. What Is the Safety Zone for Palatal Soft Tissue Graft Harvesting Based on the Locations of the Greater Palatine Artery and Foramen? A Systematic Review. *J Oral Maxillofac Surg* 2019;77:271 e271-271 e279.

11. Edel A. Clinical evaluation of free connective tissue grafts used to increase the width of keratinised gingiva. *J Clin Periodontol* 1974;1:185-196.

12. Langer B, Langer L. Subepithelial connective tissue graft technique for root coverage. *J Periodontol* 1985;56:715-720.

13. Harris RJ. The connective tissue and partial thickness double pedicle graft: a predictable method of obtaining root coverage. *J Periodontol* 1992;63:477-486.

14. Carranza N, Rojas MA. Bilaminar Palatal Connective Tissue Grafts Obtained With the Modified Double Blade Harvesting Technique: Technical Description and Case Series. *Clin Adv Periodontics* 2020;10:186-194.

15. Langer B, Calagna LJ. The subepithelial connective tissue graft. A new approach to the enhancement of anterior cosmetics. *Int J Periodontics Restorative Dent* 1982;2:22-33.

16. Hurzeler MB, Weng D. A single-incision technique to harvest subepithelial connective tissue grafts from the palate. *Int J Periodontics Restorative Dent* 1999;19:279-287.

17. Lorenzana ER, Allen EP. The single-incision palatal harvest technique: a strategy for esthetics and patient comfort. *Int J Periodontics Restorative Dent* 2000;20:297-305.

18. Bosco AF, da Silva Pereira SL, Lacerda Junior N, Milanezi LA. Análise Clínica das Áreas Doadoras de Enxerto Gengival Livre. *Revista da Associaçao Paulista de Cirurgioes-Dentistas (APCD)* 1996;50:515-521.

19. Bosco AF, Bosco JM. An alternative technique to the harvesting of a connective tissue graft from a thin palate: enhanced wound healing. *Int J Periodontics Restorative Dent* 2007;27:133-139.

20. Zucchelli G, Amore C, Sforza NM, Montebugnoli L, De Sanctis M. Bilaminar techniques for the treatment of recession-type defects. A comparative clinical study. *J Clin Periodontol* 2003;30:862-870.

21. Zucchelli G, Mele M, Stefanini M, et al. Patient morbidity and root coverage outcome after subepithelial connective tissue and de-epithelialized grafts: a comparative randomized-controlled clinical trial. *J Clin Periodontol* 2010;37:728-738.

22. Bakhishov H, Isler SC, Bozyel B, Yıldırım B, Tekindal MA, Ozdemir B. De-epithelialized gingival graft versus subepithelial connective tissue graft in the treatment of multiple adjacent gingival recessions using the tunnel technique: 1-year results of a randomized clinical trial. *J Clin Periodontol* 2021;48:970-983.

23. Zazou N, El Nahass H, Ezz El-Arab A. A technical modified method for harvesting palatal de-epithelialized connective tissue graft for root coverage: A case report. *Advanced Dent J* 2019;1:72-76.

24. Hokett SD, Peacock ME, Burns WT, Swiec GD, Cuenin MF. External root resorption following partial-thickness connective tissue graft placement: a case report. *J Periodontol* 2002;73:334-339.

25. Carnio J, Camargo PM, Kenney EB. Root resorption associated with a subepithelial connective tissue graft for root coverage: clinical and histologic report of a case. *Int J Periodontics Restorative Dent* 2003;23:391-398.

26. Wei PC, Geivelis M. A gingival cul-de-sac following a root coverage procedure with a subepithelial connective tissue submerged graft. *J Periodontol* 2003;74:1376-1380.

27. Cardoso MV, Lara VS, Sant'Ana ACP, Damante CA, Ragghianti Zangrando MS. Late complications after root coverage with two types of subepithelial connective tissue grafts, clinical and histopathological evaluation: A prospective cohort study. *J Clin Periodontol* 2021;48:431-440.

28. Parashis AO, Tatakis DN. Subepithelial connective tissue graft for root coverage: a case report of an unusual late complication of epithelial origin. *J Periodontol* 2007;78:2051-2056.

29. Breault LG, Billman MA, Lewis DM. Report of a gingival "surgical cyst" developing secondarily to a subepithelial connective tissue graft. *J Periodontol* 1997;68:392-395.

30. Wang YS, Bissonnette C, Brett C, McNamara KK, Tatakis DN. Multiple Epithelial Origin Complications Following Subepithelial Connective Tissue Graft for Root Coverage. *Clin Adv Periodontics* 2021;11:140-144.

31. Harris RJ. Formation of a cyst-like area after a connective tissue graft for root coverage. *J Periodontol* 2002;73:340-345.

32. Gil Escalante M, Tatakis DN. Gingival Cyst of the Adult as Early Sequela of Connective Tissue Grafting. *Case Rep Dent* 2015;2015:473689.

33. Romano F, Perotto S, Cricenti L, Gotti S, Aimetti M. Epithelial Inclusions Following a Bilaminar Root Coverage Procedure with a Subepithelial Connective Tissue Graft: A Histologic and Clinical Study. *Int J Periodontics Restorative Dent* 2017;37:e245-e252.

34. Corsair AJ, Iacono VJ, Moss SS. Exostosis following a subepithelial connective tissue graft. *J Int Acad Periodontol* 2001;3:38-41.

35. Lang MS, Barritt LC. Bony Exostosis After Treatment of Gingival Recession With Subepithelial Connective Tissue Graft and Enamel Matrix Derivative. *Clin Adv Periodontics* 2016;6:27-32.

36. Chambrone LA, Chambrone L. Bony exostoses developed subsequent to free gingival grafts: case series. *Br Dent J* 2005;199:146-149.

37. Roccuzzo A, Imber JC, Bosshardt D, Salvi GE, Sculean A. Development of Bone Exostosis Following the Use of a Free Gingival Graft: A 30-Year Case Report and Literature Review. *Int J Periodontics Restorative Dent* 2021;41:539-545.

38. Siegel WM, Pappas JR. Development of exostoses following skin graft vestibuloplasty: report of a case. *J Oral Maxillofac Surg* 1986;44:483-484.

39. Brouwers MC, Kerkvliet K, Spithoff K. The AGREE Reporting Checklist: a tool to improve reporting of clinical practice guidelines. *BMJ* 2016;352:i1152.

40. Demirturk Kocasarac H, Ustaoglu G, Bayrak S, et al. Evaluation of artifacts generated by titanium, zirconium, and titanium-zirconium alloy dental implants on MRI, CT, and CBCT images: A phantom study. *Oral Surg Oral Med Oral Pathol Oral Radiol* 2019;127:535-544.

41. Harris RJ. Histologic evaluation of connective tissue grafts in humans. *Int J Periodontics Restorative Dent* 2003;23:575-583.

42. Shrout PE, Fleiss JL. Intraclass correlations: uses in assessing rater reliability. *Psychol Bull* 1979;86:420-428.

43. Marques de Mattos P, Papalexiou V, Tramontina VA, et al. Evaluation of 2 techniques of epithelial removal in subepithelial connective tissue graft surgery: a comparative histological study. *J Periodontal Implant Sci* 2020;50:2-13.

44. Azar EL, Rojas MA, Patricia M, Carranza N. Histologic and Histomorphometric Analyses of De-epithelialized Free Gingival Graft in Humans. *Int J Periodontics Restorative Dent* 2019;39:221-226.

45. Sebaoun A, Cohen O, Slutzkey GS, Meir H, Nemcovsky CE, Beitlitum I. Histologic and Histomorphometric Evaluation of a Connective Tissue Graft Retrieved with a Novel Deepithelialization Procedure: A Case Series. *Int J Periodontics Restorative Dent* 2022;42:e1-e7.

46. Bertl K, Pifl M, Hirtler L, et al. Relative Composition of Fibrous Connective and Fatty/Glandular Tissue in Connective Tissue Grafts Depends on the Harvesting Technique but not the Donor Site of the Hard Palate. *J Periodontol* 2015;86:1331-1339.

47. Soehren SE, Allen AL, Cutright DE, Seibert JS. Clinical and histologic studies of donor tissues utilized for free grafts of masticatory mucosa. *J Periodontol* 1973;44:727-741.

48. Lin JC, Nevins M, Kim DM. Laser De-epithelialization of Autogenous Gingival Graft for Root Coverage and Soft Tissue Augmentation Procedures. *Int J Periodontics Restorative Dent* 2018;38:405-411.

49. Gursoy H, Yarimoglu E, Kuru B, Ozkan Karaca E, Ince Kuka G. Evaluation of the Effects of Er:YAG Laser for the De-Epithelialization of the Palatal Graft in the Treatment of Multiple Gingival Recessions: A Randomized Clinical Trial. *Photobiomodul Photomed Laser Surg* 2019;37:715-721.

50. Yoshino H, Hasuike A, Sanjo N, et al. CO(2) Laser De-epithelization Technique for Subepithelial Connective Tissue Graft: A Study of 21 Recessions. *In Vivo* 2020;34:869-875.

51. Avila-Ortiz G, Couso-Queiruga E, Pirc M, Chambrone L, Thoma DS. Outcome measures and methods of assessment of soft tissue augmentation interventions in the context of dental implant therapy: A systematic review of clinical studies published in the last 10 years. *Journal of Clinical Periodontology*;n/a.

## Figure Legends

**Figure 1**. Sequence of clinical photographs illustrating the steps followed to harvest the soft tissue sample from the posterior palate.

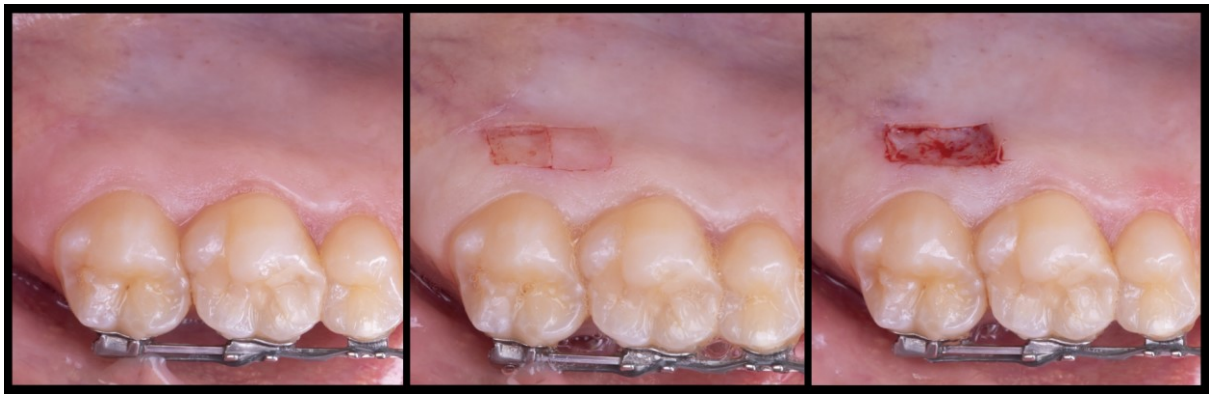

**Figure 2.** Soft tissue sample harvested from the posterior palate, after intraoral de-epithelialization but prior to extraoral epithelium removal.

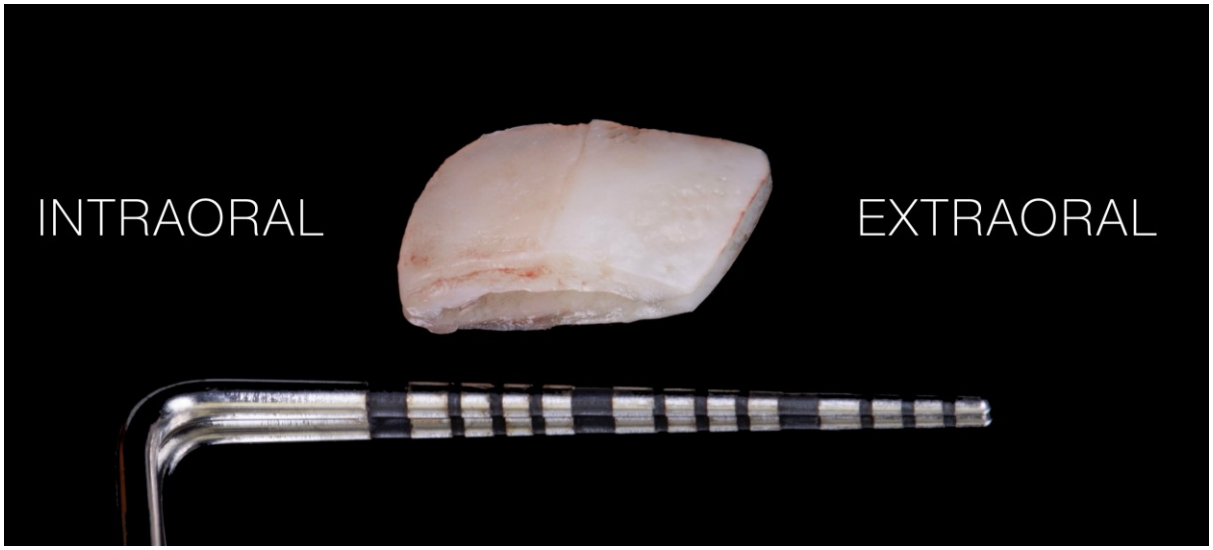

**Figure 3.** Visual depiction of the method followed for extraoral de-epithelialization of CTG samples.

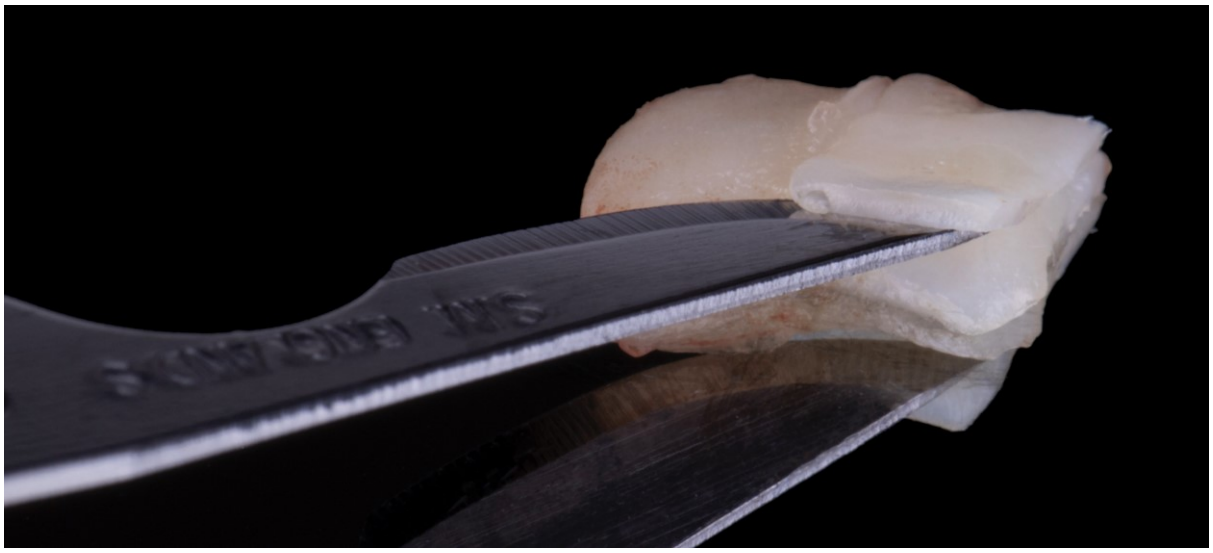

**Figure 4.** Photomicrographs of soft tissue samples (H&E staining) showing a CTG sample from the extraoral (A) and intraoral (B) de-epithelialization group.

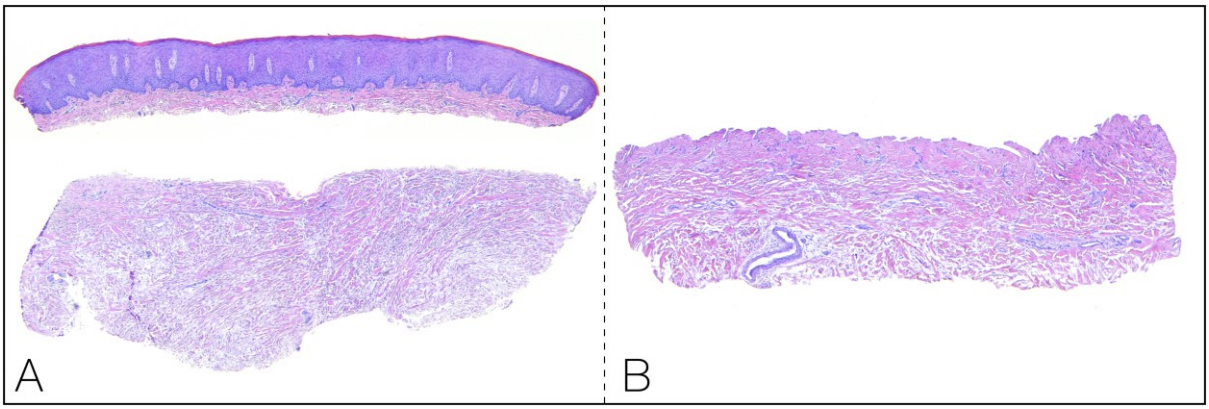

**Figure 5.** Flowchart illustrating included patients and samples.

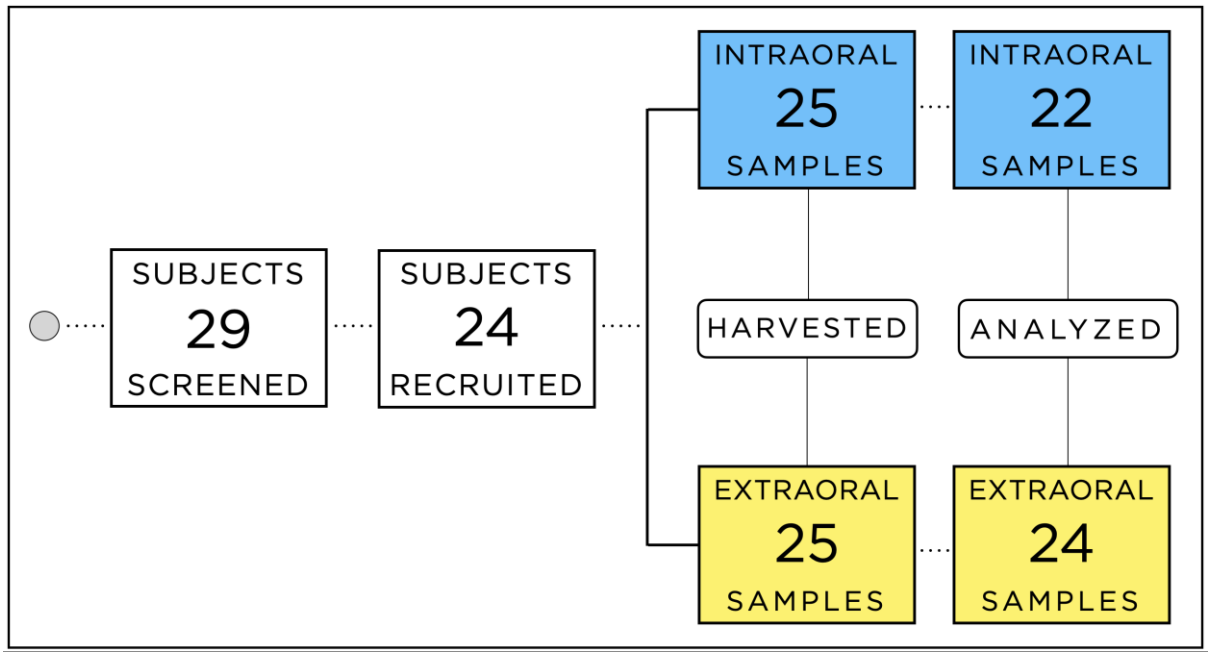


**Figure 6.** Photomicrographs of all CTG samples analyzed in this study (H&E staining). Samples were harvested from the posterior palate region and de-epithelialized extraorally (left) or intraorally (right). Samples that exhibited epithelial remnants are highlighted with a red rectangle.