

De-epithelialized connective tissue graft and the reminiscent epithelial content after harvested by the Harris' technique: a histological and morphometrical case series

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Running Title: Epithelium tissue in CTG using Harris' technique

A one-sentence summary describing the key finding: More than 50% of the CTG (i.e., Connective Tissue Graft) collected from the hard palate using the Harris' technique had the presence of epithelial tissue regardless of being harvested by an experienced surgeon.

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ABSTRACT

Introduction: The remaining epithelial layer existent in connective tissue graft (CTG) harvested from the hard palate, which underwent de-epithelization outside the oral cavity, can be histologically detected unless it is completely removed. Its presence may cause adverse esthetics results, affecting thus the color and texture of the receptor site, and an increased risk of presence of scar tissues after surgical procedures. The proposal of this study was to evaluate the CTG (histological and morphometrically) collected from the hard palate using the Harris' technique, removing the epithelial layer outside the mouth, assessing the remaining presence of epithelial tissue.

Case Series: Fourteen patients (14CTGs) were included in the present case series study, therefore there was two dropouts. A small part of the tip of the graft was harvested and fixed in formalin solution for histological processing, staining, and then to be morphometrically analyzed. The epithelial tissue and CTGs were assessed by three calibrated and double-blinded professionals. All information was compiled and performed the statistical analysis. CTGs obtained had a width average of 1224.26 μ m. There was no presence of any residual of the epithelium in three samples, whereas only one had the epithelium tissue covering the entire connective layer. Furthermore, seven samples (approximately 50%) had the presence of epithelium.

Conclusion: Within the limitation of this study, there was incomplete removal of the epithelial layer after harvesting the CTG using the Harris' technique (44.32%), most likely due to its histological persistency, suggesting to be inaccurate the clinical removal.

Keywords: Connective tissue; Epithelium; Histology; Periodontics; Plastic surgery; Tissue grafts.

BACKGROUND

The treatment of gingival recession (GR) is an approach found within the periodontal plastic surgery (PPS)¹, which has several techniques described in the literature for the treatment of gingival recession (GR)², effectively enabling the

reduction of GR and increasing levels of great clinical achievements³. In this context, the Connective Tissue Graft (CTG) has been largely used in GR treatments⁴, showing superior efficacy³ and satisfactory aesthetic results⁵.

Harris developed a procedure using a double blade with two parallel and concomitant incisions to get access to the CTG⁶, which was considered an ideal technique⁷ with satisfactory results, respecting the anatomical and biological limitations⁸, and avoiding major damages. However, one of the adverse clinical issues associated with CTG can be the presence of remnant epithelium layer, which may not be completely removed from the CTG before the insertion⁹, impairing thus the esthetic color and texture. Also, an increased risk of postoperative scarring tissue formation can occur¹⁰, preventing adequate healing and tissue integration. Furthermore, epithelial presence in soft tissue grafts could be associated with cystic lesions, such as demonstrated in the histopathological observation in the Escalante and Tatakis' study¹¹, which found a stratified squamous nonkeratinized cystic epithelium.

In this fashion, how to adequately remove the histologic epithelial tissue before insertion remains unclear³. Thus, this study evaluated histologically and morphometrically the presence of a reminiscent epithelium layer after the CTGs have been harvested using the Harris' technique, following its original description, and removing the epithelial layer outside the mouth.

CLINICAL PRESENTATION

This study followed the Declaration of Helsinki (1964, updated 2013), and it was approved by the Ethics Committee (60559516.0.0000.5291). After explaining the procedure to the patients and having a written formal agreement, CTGs were harvested in a private practice environment (Rio de Janeiro, Brazil), between February 2018 and January 2019, by the same experienced surgeon (S.K.). Patients were under treatment of RC, showing a state of good health and any contraindications to surgical periodontal therapy, such as poor standard of plaque control, questionable long-term prognosis of patient dentition, pregnancy, smoking, severe cardiovascular disease, malignancy, bleeding disorders, uncontrolled diabetes.

The exclusion criteria utilized were followed by uncontrolled systemic disease or inadequate plaque control, and whether the patient was a smoker; while the inclusion criteria was based on the GR Miller's classification¹² (i.e., presence of class I or II, but currently RT 1)¹³, whose patients had the necessity of an RC procedure.

CASE MANAGEMENT

Harris' technique description⁶

The standard technique uses a scalpel with double-parallel blades to obtain a pair of parallel incisions in the palate, staying at least 2 mm away from the gingival margin. These incisions were extended for 10 to 12 mm medially into the palate. It is suggested vertical releasing incisions when necessary. The result is a uniform thickness piece of tissue, composed predominantly by connective tissue, with an epithelial border. Then, this epithelium is removed and discarded and pressure should be applied, with a wet gauze, to the donor area.

Surgical and histological procedures

Fourteen subjects, both genders, were included in this study (mean age 37 years old, ranging between 25 and 54 years old). All surgical procedures were performed using two 15c blades and a periodontal probe for the Harris' technique^{6,7}, following next to the sequential removal of the epithelium layer, extraorally, using another scalpel and blade 15c. The standard site for the harvesting of CTG was between the distal surface of the canine and distal surface of the first molar, with a 2 to 3 mm distance far from the palatal gingival margin, avoiding thus potential histologic variability⁹, which can influence not only the volume stability of the graft but also the process of the graft revascularization¹⁰.

For the histological procedures, a small part of the tip of the tissue collected (Figure 2) from the tissue graft harvested (2 mm measured with a periodontal probe), and were fixed in microtubes of 0.5 mL using a 10% buffered formalin solution (1.27mol/L formaldehyde in 0.1 M phosphate buffer, pH=7.2) for a period of 48 hours at room temperature. Afterwards, these materials were dehydrated in a series of alcohol baths with progressive and increasing concentrations (e.g., 50%, 70%, 90%, and 95% concentrations for 10 minutes/each) until reaching the concentration of the absolute alcohol. Further, they were diaphanized in Xylol (3 baths for 45

minutes/each) and added in a Paraplast Plus^{||}, following standard techniques. The pieces were sectioned with 3 μm thickness and afterwards stained in hematoxylin and eosin (HE).

Morphological and Morphometric Analysis

The histological slides were completely evaluated using a light microscope and a digital camera[¶]. Pictures were taken to allow the morphological evaluation of the integrity of epidermal ridges, the dermal papillae, and the epithelial extension (Table 1, Figure 1). The images were measured morphometrically[#] (Figure 3) and considering the presence or absence of epithelial tissue. All images were assessed independently by two calibrated and double-blinded professionals (executed by V.T.G.M. and A.B.S.), and the opinion of a third referee (G.V.O.F.) was also independently consulted.

CLINICAL OUTCOMES

Two of the CTGs samples were excluded from our analysis due to impracticable reason (loss of the material after attempt to perform the histological inclusion). CTGs dimensions and the reminiscent epithelial tissue were thus measured (Figure 4). CTGs obtained here had an average of 1224.26 μm (± 179.13 μm), with a minimum and maximum dimension of, respectively, 989.47 μm and 1610.88 μm .

For the morphometrical evaluation, the measures were detailed in Table 2. There was no presence of any residual epithelium layer in three of our samples (25%), whereas only one sample (8.33%) had the epithelium tissue covering all connective layers (Table 2, Figure 3). Samples showing a lack of epithelium tissue were in average 55.68% of our samples ($\pm 32.95\%$), whereas its presence was of 44.32% ($\pm 32.94\%$).

Seven samples (58.33%) showed the presence of the epithelium tissue. The epidermal ridges were completely absent in three samples, which had the epithelium completely removed, while the other nine samples had the presence of the

^{||} (Sigma-Aldrich, USA)

[¶] (LC Evolution, Olympus BX 51 microscope, USA)

[#] (Software Image-Pro Plus v. 8.0, Media Cybernetics, USA)

epidermal ridges. The dermal papillae were found in all CTGs samples, but sometimes part of this structure was removed.

DISCUSSION

Previous studies^{8,9} have demonstrated excellent results for the application of CTG in RC procedures. Likewise, Harris¹⁴ have published a study case in which had 6.5 mm of recession on a molar tooth, resulting in a coverage of 84.6%. Other studies¹⁵ have reported a greater gain in width of keratinized tissue and mean RC (98.4%) when the Harris' technique was applied¹³. However, just a few histological studies have been found in the literature^{9,16}, and there is practically no study so far that have evaluated the dimensions of the remnant epithelial layer.

Hence, this study utilizing the Harris' technique^{6,7} obtained a dimensional CTG average of 1224.26µm and an overall average for the epithelium presence of 44.32%. Comparing with a previous study⁹, it was verified the presence of epithelium in 80% of the specimens analyzed, the presence of lamina propria (mean of 65.2%), and sometimes a great portion of adipose tissue was present as well. According to Maurer *et al.*¹⁷, the submucosal (adipose) and the epithelial tissues should be completely removed to avoid complications when proceeding with the induction of new keratinized tissue. In this study, three samples (25%) did not have any epithelium residues, contrasting with one sample (8.33%) in which the epithelium has completely covered the connective layer.

The epithelial layer may cause differences in local color, texture, an increased risk of scarring tissue, and preventing an adequate healing process⁸, which may cause sequelae in the receptor site¹⁶. Therefore, many of those results could be not significant, either for patients as for professionals, due to the requirement level of both to be low, beyond could also be non-significant to reach a scientific publication. In addition, even though when the graft is completely covered by the flap, might appear a lighter shade in the local tissue after healing period. For this reason, Zuhr *et al.*¹⁰ suggested the inversion of the superficial side of the graft inwards rather than outwards in the recipient bed. Moreover, there is an elevated risk associated with the epithelium presence, which may cause an epithelial cyst¹¹. A rational conclusion could be that the epithelial layer might cause a negative morphogenetic stimulus.

Conclusion

Thus, within the limitation of this study, it was possible to conclude that there was incomplete removal of the epithelium layer due to its histological presence in 44.32% of the samples, after to apply the Harris' technique to harvest the CTG, suggesting that the clinical removal of the reminiscent epithelium layer may be inaccurate.

Summary

<ul style="list-style-type: none"> • Why is this case series new information? 	<p>This study demonstrated that the presence of a reminiscent epithelium layer might cause adverse and undesirable clinical responses after a CTG insertion in periodontal and peri-implant regenerations.</p>
<ul style="list-style-type: none"> • What are the keys to successful management of this case? 	<p>There is still an epithelial presence close to the CTG, which is recommended to be completely removed.</p>
<ul style="list-style-type: none"> • What are the primary limitations for the success in this case? 	<p>Great difficult to remove clinically the reminiscent epithelium layer once they are strongly associated to the connective tissue.</p>

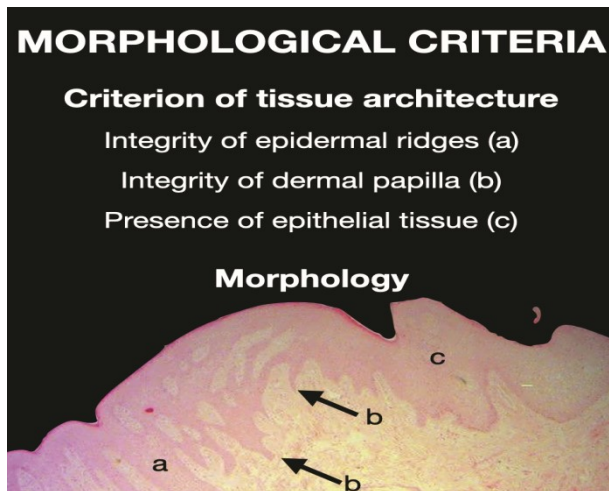
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FIGURE LEGENDS

Figure 1. Scheme of the criteria used for morphometric analysis of connective tissue graft used surgically and collected through the Harris technique (double blade).



(Exemplary Image) Figure 2. A. Epithelial and connective tissue harvested from the hard palate; B. Extraoral de-epithelization using a scalpel and one blade 15c; C. Section collected from the tip of the graft for histologic and morphometrical analysis.

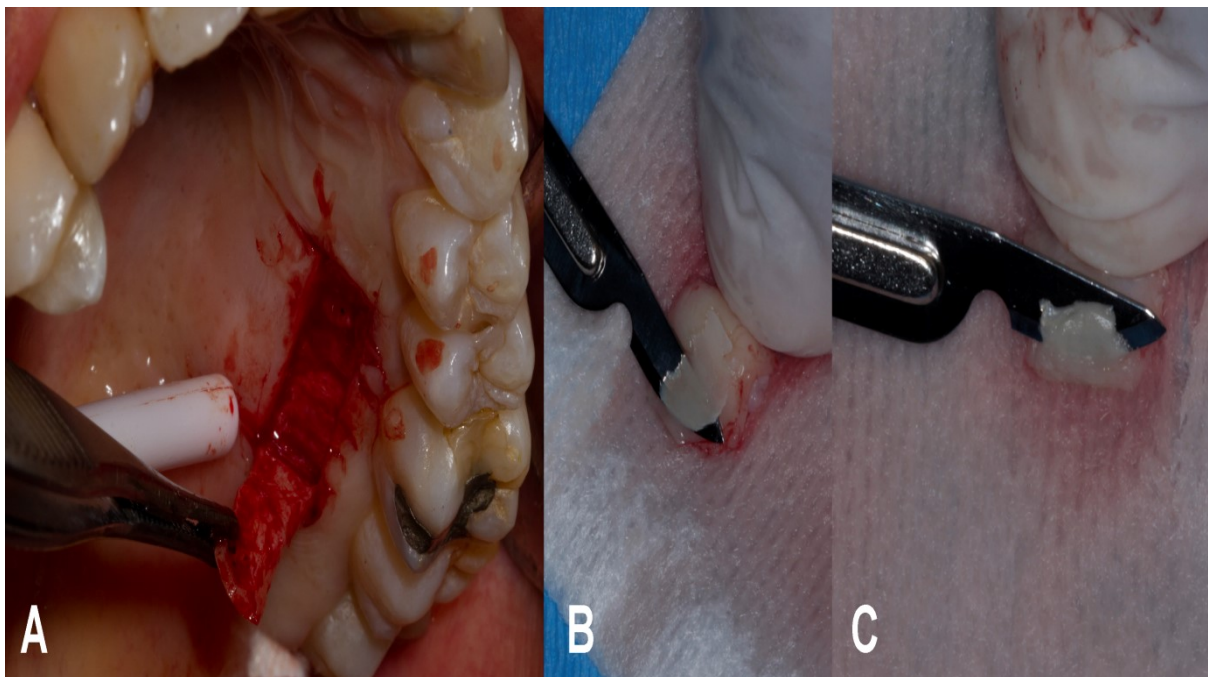
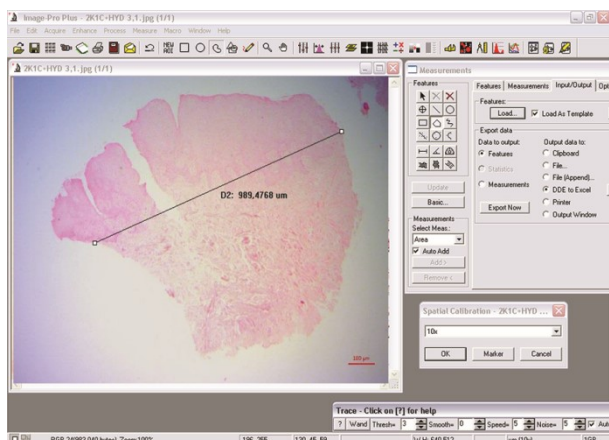
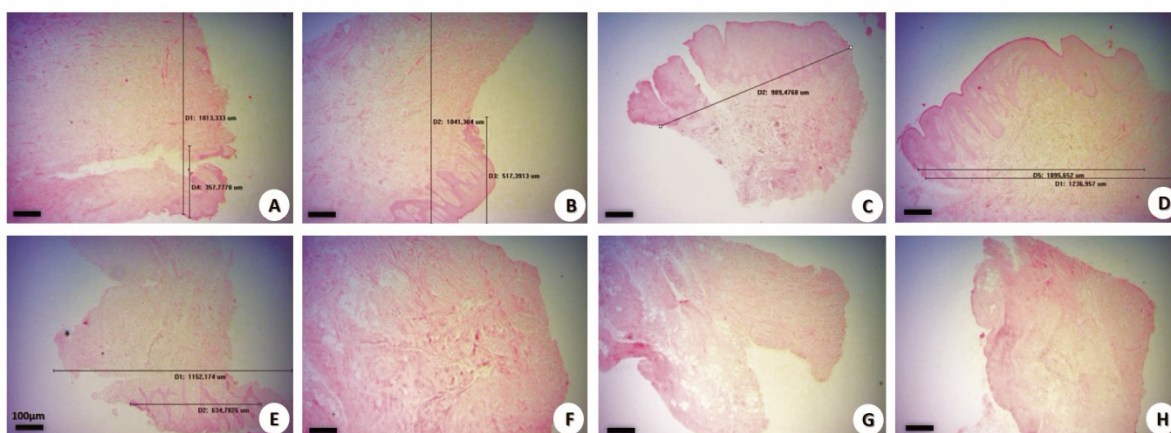


Figure 3. Morphometric analysis scheme performed with the Image-Pro Plus software (version 8.0, Media Cybernetics, Silver Spring, MD, USA).



(Exemplary Image) Figure 4. Photomicrographs of the connective tissue graft harvested using Harris technique. The blades of the samples were stained with Hematoxylin and Eosin (HE). A-E. Length of the connective tissue obtained. F. A total absence of the epithelial layer; G-H. Presence of the epithelial layer.



CONFLICTS OF INTEREST: The authors declare that there are no conflicts of interest associated with this study.

Table 1. Criteria utilized for morphometric analysis using the Harris Technique to harvest the CTG

HISTOMORPHOMETRIC ANALYSIS	
Morphology	Morphometry
1. Epidermis Crests Integrity	1. Major width of the CTG
2. Dermis Papilla Integrity	2. Major width of the CTG with Epithelial Tissue
3. Epithelial Tissue Presence	3. Percentual (%) of the lack of Epithelium

Table 2. Morphometric results of the CTG collected using the Harris Technique

Sample	Larger CTG width (μm)	CTG width <u>with</u> Epithelium (μm)	Lack of Epithelium (%)
#1	1013.33	357.77	64.69
#2	1401.28	0	100.00
#3	1334.22	0	100.00
#4	989.47	989.48	0
#5	1610.88	554.38	65.58
#6	1152.17	634.78	44.91
#7	1305.99	0	100.00
#8	1108.18	754.30	31.93
#9	1237.25	576.61	53.40
#10	1260.04	681.89	45.88
#11	1236.96	1095.65	11.42
#12	1041.30	517.39	50.31