Histologic and Histomorphometric Analyses of De-epithelialized Free Gingival Graft in Humans

Emilio L. Azar, DDS1
Mariana A. Rojas, DDS1
Mandalunis Patricia, BSc, PhD2
Nelson Carranza, DDS, MS, PhD3

A graft’s histologic composition depends on the harvesting technique used, and different connective tissue–harvesting procedures have been described in the literature. Some authors suggest the submucosal tissue not be incorporated into the graft because it may interfere with the graft revascularization. In those cases, the de-epithelialized gingival graft (DGG) is obtained with a superficial harvesting technique that leaves the deep portion of the submucosa and the periosteum excluded from the graft. The aim of this case series was to histologically and histomorphometrically evaluate the tissue obtained with this technique. The findings demonstrated that the DGG was mainly composed of connective tissue, and adipose tissue was in minimal proportions. However, epithelium was found in all of the samples. Int J Periodontics Restorative Dent 2019;39:221–226. doi: 10.11607/prd.3544

Different techniques for harvesting connective tissue graft (CTG) have been described in the literature, and the evaluation of the palatal thickness is critical. In some clinical situations, the tissue is not thick enough for the primary flap and the graft, resulting in a greater risk of incorporated fatty and glandular tissues in the graft; it has been suggested that this tissue be removed because it can interfere with the graft’s revascularization. Ouhayoun et al, in a histologic and biochemical study in humans, suggested that the deep portion of the connective tissue from the palate will not induce keratinization. According to Zucchelli et al, traditional CTG harvesting techniques are not recommended if the palatal soft tissue is not sufficiently thick because of the risk of primary flap necrosis and/or the inadequacy of the graft (due to the presence of a fatty and glandular tissue instead of a desirable connective tissue). In such cases, a de-epithelialized free gingival graft (DGG) technique was recommended. In the same study, the authors compared CTG with DGG for the treatment of gingival recessions and observed a greater increase in gingival thickness at the buccal aspects of the patients treated with DGG, even though no differences were found in the thickness of the graft at the time of suturing between the two treatment

1Department of Periodontics, University of Buenos Aires, Buenos Aires, Argentina.
2Department of Histology and Embriology, University of Buenos Aires, Buenos Aires, Argentina.
3Private Practice, Carranza Institute, Buenos Aires; Department of Periodontics, University of Buenos Aires, Buenos Aires, Argentina.

Correspondence to: Dr Mariana Andrea Rojas, Department of Periodontics, University of Buenos Aires, Marcelo T de Alvear 2142 C1122AAH CABA, Buenos Aires, Argentina. Email: rojasmarianaaandrea@gmail.com

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groups. Zucchelli et al speculated that the differences in the quality of the connective tissues used in the two techniques were responsible for this increase in gingival thickness because DGG allows the incorporation of the portion of connective tissue closest to the epithelium into the graft. This tissue is dense, firmer, more stable, and presumably more suitable for root coverage. Therefore, the DGG is a harvesting technique designed to leave the deep portion of the submucosa and the periosteum excluded from the graft. The authors believe that the proportion of adipose tissue obtained with this technique is minimal, but until the present and to the best of the authors’ knowledge, there has not been a histologic study in humans that describes the composition of the CTG harvested with this technique only, and the need is critical. Therefore, the purpose of this case series was to histologically and histomorphometrically evaluate the characteristics of DGG in its final stage of preparation, immediately before its application.

Materials and Methods

Five healthy adults were selected from a pool of patients referred to the Department of Periodontology, School of Dentistry, University of Buenos Aires for various procedures that required CTG. Two cases of biotype thickening in implants and three cases of root coverage in patients, with multiple adjacent Miller Class I gingival recession defects, were treated (Figs 1 to 9).

Surgical Procedure: Sample Collection

All grafts were taken from the maxillary palatal area, ranging from the canine to the first molar, and samples were taken from between the second...
premolar and first molar. The surgical technique was performed following the description by Zucchelli et al:

“... Two horizontal (the coronal incision was performed 1–1.5 mm apical to the soft tissue margin of the adjacent teeth) and two vertical incisions were traced to delimitate the area to be grafted. Along the coronal incision, the blade was oriented almost perpendicular to the bone plate and once an adequate soft tissue was obtained, it was rotated in order to be almost parallel to the superficial surface. The thickness of the graft was maintained uniform at approximately 1.5 mm, while proceeding apically with the blade. The graft was de-epithelialized with a 15c blade.”

The de-epithelization of the graft was performed using a surgical microscope (×10 magnification), utilizing the reflection of light as a reference to determine the complete removal of the epithelium. After epithelial removal, the thickness of the grafts was reduced to approximately 0.8 mm (Fig 2). The histologic sample was obtained by removing a 2-mm-wide segment from the most distal end of the graft (Fig 3). All samples were immediately fixed in 10% formalin for histologic analysis.

Histologic Processing

Histologic samples were processed at the Department of Histology, University of Buenos Aires. Sections measuring 7 μm thick were prepared and stained with hematoxylin-eosin.

Sample Analysis

All sections were first scanned with a microscope (Axio Lab.A1, ZEISS) using a digital microscopy system (ZEN blue edition 2011, ZEISS). Digital JPEG images were obtained. When the sample size was larger than the microscope’s field of vision, a series of microphotographs were obtained without altering the magnification and focus.

Tissue-composition analysis was performed on the microphotograph with image analysis software (Image Pro Plus, Media Cybernetics), outlining the following areas:

- Connective tissue proper area (CT/TA [%]): Fraction of total area corresponding to connective tissue proper area
- Adipose tissue area (AT/TA [%]): Fraction of total area corresponding to adipose tissue area
- Vascular tissue area (VT/TA [%]): Fraction of total area corresponding to vascular tissue area
- Epithelial tissue area (ET/TA [%]): Fraction of total area corresponding to epithelial tissue area
Results

Histomorphometric results are presented in Table 1. The complete sample and representative sections are shown in Figs 10 and 11. The most notable feature of the samples was their homogeneity (Fig 10b). The samples were composed by dense connective tissue (CT, median 89.17% of the total area) with minimal amount of adipose tissue (AT, median 1.11% of the total area). Vascular tissue (VT) was found in minimal proportions (< 3% of the total area) in all samples except one, where VT was observed in 11.24% of the total area (Fig 11). Remnants of epithelial tissue (ET) were found in different proportions among all the samples (median 6.01% of the total area, Figs 10 and 11).

All of the grafts were adequate in size and volume for their intended applications, and they produced clinically successful results. The surgical procedures were tolerated well, without complications. The healing patterns of the palatal wounds were similar for all treated cases. An example of the healing response at 1-week postsurgery is shown in Fig 8. The level of discomfort and pain reported by the patients was described as minimal.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Sample 1</th>
<th>Sample 2</th>
<th>Sample 3</th>
<th>Sample 4</th>
<th>Sample 5</th>
<th>Median</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>CT (% of TA)</td>
<td>89.17</td>
<td>89.33</td>
<td>86.53</td>
<td>78.66</td>
<td>92.20</td>
<td>89.17</td>
<td>78.66–92.20</td>
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<tr>
<td>AT (% of TA)</td>
<td>1.11</td>
<td>4.70</td>
<td>0.00</td>
<td>4.08</td>
<td>0.85</td>
<td>1.11</td>
<td>0.00–4.70</td>
</tr>
<tr>
<td>VT (% of TA)</td>
<td>1.50</td>
<td>2.73</td>
<td>1.01</td>
<td>11.24</td>
<td>2.59</td>
<td>2.73</td>
<td>1.01–11.24</td>
</tr>
<tr>
<td>ET (% of TA)</td>
<td>8.22</td>
<td>3.23</td>
<td>12.46</td>
<td>6.01</td>
<td>4.36</td>
<td>6.01</td>
<td>3.23–12.46</td>
</tr>
</tbody>
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CT = connective tissue; TA = total area; AT = adipose tissue; VT = vascular tissue; ET = epithelial tissue.

Fig 10 (left) Sample 2. (a) Predominantly dense connective tissue was observed with some epithelial and adipose tissues (H&E stain; original magnification ×25). (b) Apical portion. Note the epithelium still present. Dense connective tissue with some blood vessels and adipocytes were observed (H&E stain; original magnification ×100).

Fig 11 (right) Sample 4. (a) Dense connective tissue. Minimal amount of epithelium and absence of adipose tissue was observed. (H&E stain; original magnification ×25). (b) Central portion. Dense connective tissue was observed. Note the absence of epithelium (H&E stain; original magnification ×100).
Discussion

The aim of the present case series was to analyze the histologic characteristics of a completed DGG graft immediately before it was applied to the recipient site, describing and quantifying its tissue components. The histologic and histomorphometric analyses of the graft harvested in humans using this technique were not reported previously.

In a histologic study in fresh human cadavers, Bertl et al. evaluated the composition of the anterior and posterior palatal mucosae and observed that higher amounts of adipose tissue and lower amounts of dense connective tissue are found in areas where CTGs are usually harvested with split-flap techniques than in more superficial areas where de-epithelialized grafts are harvested. Another recent histologic and immunohistochemical study also described the composition of the harvesting area of the palatal mucosa. The authors reported less cellular components with larger blood vessels in deeper connective tissue than on the surface. The results of the present case series demonstrated that a CTG obtained with the DGG technique is mainly composed of dense CT (more than 75% of the total area in all the samples; median 89.17% of the total area, range 78.66% to 92.20%). It was also observed that adipose tissue was present in minimal amounts (median 1.11% of the total area, range 0.00% to 4.70%), confirming the adequacy of the DGG harvesting technique towards this goal. Although previous studies suggest that CT can interfere with the revascularization of the graft and impede keratinization, the clinical relevance of that finding remains to be confirmed.

Despite the efforts to carefully remove the ET, the results show that small remnants were present in all samples in different proportions (median 6.01% of the total area, range 3.23% to 12.46%). It has been suggested that presence of the epithelium on the graft may result in complications, such as epithelial cysts and edema. However, several authors suggested leaving residual epithelium on the graft and did not report any problems. These observations coincide with the present results, since the inclusion of the epithelium did not seem to affect the clinical results of the present cases. Harris, in his histologic study, arrived at similar conclusions.

When the samples were observed after epithelium removal, it was necessary to reduce the graft thickness by almost 50%. Although no attempts were made to harvest and evaluate thicker grafts, the fact that some residual fatty tissue could be seen in some samples, together with the information from published histologic analyses of palatal tissue, suggests that harvesting thicker grafts would only render larger proportions of submucosal tissue.

Thus, DGG thickness is likely limited by the available subepithelial lamina propria, predictably rendering grafts of less than 1 mm. If thicker grafts are desired, it is likely that fatty and glandular tissues must be included in the graft. The obtained graft thickness of approximately 0.8 mm has been successfully reported to be suitable for root coverage procedures. However, it remains to be established if DGGs can be successfully applied to clinical situations where more volume is required, such as ridge augmentations or biotype thickening. In the present study, three cases of root coverage and two cases of biotype thickening in implants were successfully treated. The samples examined in this study were obtained from the distal ends of grafts, all corresponding to a small area located between the second premolar and the first molar. Bertl et al. showed that the thickness of the ET and its lamina propria were constant between the anterior and posterior palate. It is probable, then, that the observations reported from these samples also apply to grafts obtained from more anterior and more posterior areas.

Finally, it must be considered that short- and long-term success has been extensively reported in root-coverage and tissue biotype-thickening procedures that utilized traditional harvesting techniques, obtaining CTGs as well as submucosal (called “undesirable”) tissues.

Conclusions

Within the limitations of the present five-case series, the DGG technique was found to be simple and applicable to different clinical situations, with minimal morbidity and no postsurgical complications. The histologic results showed that this graft could be described as a “predominantly dense CTG” since minimal
amounts of adipose and epithelial tissues were found. Implications of these tissue remnants should be further evaluated in larger size in long-term clinical and histologic studies.

Acknowledgments

The authors declare no conflicts of interest.

References