Histologic and Histomorphometric Evaluation of a Connective Tissue Graft Retrieved with a Novel Deepithelialization Procedure: A Case Series

Various free connective tissue graft (CTG) harvesting techniques have been reported. The lining epithelium of the palatal graft may be retrieved either intra- or extraorally. This report presents a series of root coverage cases where deepithelialization was intraorally performed before harvesting the graft with a round diamond bur mounted on a low-speed handpiece. Ten single-tooth gingival recession defects in five patients were treated, applying a surgical procedure based on a coronally advanced flap combined with a free CTG that was deepithelialized in situ by the same method. Recession and probing depths and keratinized tissue and recession widths were recorded at baseline and the follow-up evaluations. Follow-up was between 7 and 21 months (mean: 12.1 ± 5.04 months). Clinical, esthetic, and histologic evaluations were performed. Mean root coverage was 89% ± 24.86% (range: 25% to 100%), and complete root coverage was observed in 80% of cases; the esthetic score range was 6 to 9 (mean: 7.44 ± 1.01). Epithelial remnants, although different in proportions, were evident in all samples (range of prevalence: 4.57% to 29.12%). Within the limitations of the small number of clinical cases, the presented in situ deepithelialization technique for CTG seems to be valuable and may accordingly be routinely applied. Int J Periodontics Restorative Dent 2022;42:e1–e7. doi: 10.11607/prd.5536

Connective tissue graft (CTG) is extensively applied in periodontal plastic surgery for root coverage, correction of edentulous ridge defects, and implant-supported rehabilitation procedures. In root coverage procedures, free CTGs are considered the gold standard and, in conjunction with coronally advanced flap (CAF), are accepted as the most effective and predictable method for gingival recession treatment. There are two main donor sites for CTG harvesting: the most frequently used is the lateral palate, and the tuberosity is the second option. Grafts are harvested from the tuberosity by distal wedge or gingivectomy procedures, requiring extraoral graft deepithelialization.

Harvesting techniques intending to retrieve the deep layers of connective tissue may cause over-thinning of the palatal flap, leading to sloughing with secondary intention healing, especially when vertical incisions are performed, thus increasing patient morbidity. In a histologic study, Harris showed that CTGs retrieved from the deep palate differ between individuals—While the entire graft was composed of lamina propria and contained no submucosa or adipose tissue in some instances, these were the main components in others. Superficial layers of the palatal connective
tissue have been reported to have a higher potential for keratinization induction than the deep layers,\textsuperscript{14,15} also presenting a larger amount of fatty glandular tissue (FGT),\textsuperscript{15} which could possibly act as a barrier to plasmatic circulation and impair revascularization during the early healing phase.\textsuperscript{16} Therefore, traditional CTG harvesting techniques are not recommended if the palatal soft tissue is not thick enough.\textsuperscript{5} Zucchelli et al\textsuperscript{5} described a CTG harvesting technique from the superficial palatal tissues, excluding the deep portion of the submucosa and the periosteum. A free gingival graft (FGG) is harvested and then extraorally deepithelialized with a 15C blade. This approach showed similar postoperative morbidity compared to the traditional one,\textsuperscript{5} but it is easier to perform, even where thin palatal tissues are present.\textsuperscript{5,17} Deepithelialized CTG consists mainly of lamina propria that contains more fibrous connective tissue and less FGT compared to subepithelial CTG (SCTG), which consists mainly of submucosa,\textsuperscript{18} thus obtaining dense, firm, and stable CTGs. In a recent histologic and histomorphometric analysis of deepithelialized FGGs in humans, remnants of epithelium were seen in different proportions in all examined samples.\textsuperscript{19} However, inclusion of epithelium in the graft did not seem to affect the root coverage procedure’s clinical outcome.\textsuperscript{13,19} In a case report where CAF+CTG was applied for root coverage, CTG was deepithelialized in situ and then harvested with a high-speed, mounted, round diamond bur.\textsuperscript{20} The purpose of this paper is to present a series of root coverage cases where deep epithelialization was performed before harvesting the graft using round, diamond burs mounted on a low-speed handpiece. Clinical outcomes of the procedures are presented together with histologic analysis of the free grafts.

Materials and Methods

Five healthy, nonsmoking, adult patients (four women, one man) aged 21 to 32 years (mean: 23 ± 3.2 years) were treated by the same periodontist (A.S.). This case series comprised 10 gingival recessions treated by applying surgical procedures based on a CAF combined with a free CTG, all deepithelialized by the same method. All recessions were Miller Class I or II\textsuperscript{21}/recession type (RT) 1 according to Cairo et al.\textsuperscript{22} In four patients, single recessions were separately treated (six in the mandible and two in the maxilla), while in one patient, two adjacent recessions in the mandible were treated. All patients signed an informed consent form concerning the planned treatment.

At baseline, all subjects received detailed information concerning the mucogingival defect, including etiology. Detailed oral hygiene instructions were provided, traumatic tooth brushing was eliminated, and scaling and root planing were indicated and performed.

The clinical evaluation was not blinded, as the same operator (A.S.) performed all surgical procedures and clinical recordings. The following parameters were recorded at the baseline surgical appointment:

- Recession depth (RD), measured from the cementoenamel junction (CEJ) to the gingival margin at the midfacial aspect of the tooth
- Probing depth (PD), measured at the midfacial aspect of the recession, from the gingival margin to the bottom of the pocket
- Keratinized tissue width (KT), measured at the midfacial aspect of the tooth, from the gingival margin to the mucogingival junction (MGJ)
- Recession width (RW), measured at the CEJ level

All measurements were performed with a North Carolina periodontal probe (Hu-Friedy) and rounded to the nearest millimeter.

The exposed root surfaces were planed with Gracey curettes (SAS 1-2 or SMS 1-2, Hu-Friedy) and thoroughly scrubbed and rinsed with saline.

Surgical Procedure and Sample Collection

In all cases, the donor site was the lateral palate in the premolar to second molar region.\textsuperscript{23} Two horizontal and two vertical incisions were made with a no. 15 blade, involving only the epithelium and the superficial underlying connective tissue. The incisions delimited a quadrangular area slightly larger
than that needed for the root coverage procedure.

First, deepithelialization of the future graft was performed with a large, coarse-grit, round diamond bur, (A5L, Strauss) mounted on a surgical contra-angle handpiece with 45-degree head (WS-91 L, WH) on an implant motor (Implantmed, WH) at 4,000 rpm with copious sterile saline irrigation. Then, a similar, fine-grit bur replaced the previous coarse-grit bur to achieve a smooth and uniform tissue surface. Bleeding at the donor area surface served as a clinical indicator of deepithelialization (Fig 1a). Using a no. 15 blade, a uniform graft approximately 1 mm thick was dissected, applying Zucchelli et al’s technique. The retrieved tissue was then transferred and sutured at the recipient site. Sutures were applied at the donor site, and the palatal wound was protected with a collagen resorbable sponge (Collafleece, Botiss Biomaterials). Periodontal dressing (Coe-Pak, GC America) was applied over the collagen sponge, protecting the donor site.

The precise root coverage procedure for each case was arbitrarily selected by the surgeon (Figs 1b to 1g and 2). However, all of the procedures were variations of CAF + free CTG and were well documented. All surgical work was performed with the aid of magnification loupes (x4.5) with an illumination source.
Esthetic evaluation of the treated recessions was done by a blind experienced periodontist (G.S.S.) using the root coverage esthetic score by Cairo et al. The esthetic evaluation was done using pre- and posttreatment pictures (taken between 7 and 21 months posttreatment).

Histologic samples were obtained by removing a 2-mm–wide segment from one of the graft edges (mesial or distal, selected arbitrarily) using a no. 15 blade. Samples were fixed in 10% formalin and processed for histologic analysis.

**Histologic Processing and Sample Analysis**

Histologic samples were processed at the Department of Oral Biology, Tel-Aviv University. Tissue samples were embedded in paraffin, blocks were prepared, and 6-µm sections were mounted and stained with Mallory’s trichrome (Figs 1g and 2g).

Stained sections were photographed with a digital camera (AxioCam MRc, Carl Zeiss) mounted on a light microscope (Axio Imager M2, Carl Zeiss), and digital images (×4 magnification) of the whole section were obtained. Histomorphometric analysis of the microphotographs was performed with Photoshop CS6 extended software (Adobe). The following areas were outlined and calculated: total tissue

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**Fig 2** (a) Deepithelialized donor site before graft harvesting. (b) Preoperative view. A 4-mm gingival recession is evident on the maxillary left canine. (c) The CTG was sutured at the recipient site. (d) A coronally displaced double pedicle graft completely covers the CTG and the cementoenamel junction. (e) Clinical views at the 2-month and (f) 14-month follow-ups. Note the preserved complete root coverage. (g) Histologic section (×4 magnification) stained with Mallory’s trichrome. The sample is mainly comprised of connective tissue (mostly stained in blue), with some epithelial remnants (stained in red) comprising 8.57% of the area.
surface area, total epithelial rests surface, and the percentage of epithelial rests surface out of the total surface.

Results

Postoperative healing was uneventful for 9 out of 10 treated recessions except one. In 1 treated recession, a retreatment procedure was needed due to early partial failure.

With the exception of the early partial failure case, differences between pre- and postoperative values of all parameters were statistically significant in all recession defect procedures.

Mean root coverage for the 10 treated recessions was 89% ± 24.86% (range: 25% to 100%). Complete root coverage was achieved in 80% of recession sites (8 of 10). KT gain was 3 ± 0.9 mm (range: 1 to 4 mm). Follow-up time ranged from 7 to 21 months (mean: 12.1 ± 5.04 months). Results are summarized in Table 1.

The esthetic score (according to Cairo et al\(^2\)) of the treated recessions was between 7 and 9 (mean: 7.44 ± 1.01) for all but one partial failure case (score of 1) and two recessions where a keloid-like appearance was observed (score of 6 for each).

The palatal donor site healed without any complications, unusual discomfort, and/or bleeding.

Histologic Evaluation

Histomorphometric results are presented in Table 2. The most notable

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**Table 1** Recession Depth and Keratinized Tissue Width Before and After Root Coverage with Corresponding Esthetic Evaluation

<table>
<thead>
<tr>
<th>Site no.</th>
<th>Recession depth (mm)</th>
<th>KT width (mm)</th>
<th>Esthetic score</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>Baseline</td>
<td>Follow-up</td>
<td>RecRed</td>
</tr>
<tr>
<td>1</td>
<td>4</td>
<td>0</td>
<td>4</td>
</tr>
<tr>
<td>2</td>
<td>4</td>
<td>0</td>
<td>4</td>
</tr>
<tr>
<td>3</td>
<td>4</td>
<td>0</td>
<td>4</td>
</tr>
<tr>
<td>4</td>
<td>4</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
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<td>3</td>
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</tr>
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<td>0</td>
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<td>1</td>
<td>2</td>
</tr>
<tr>
<td>10</td>
<td>4</td>
<td>0</td>
<td>4</td>
</tr>
</tbody>
</table>

KT = keratinized tissue; RecRed = recession reduction.

**Table 2** Composition of Grafts

<table>
<thead>
<tr>
<th>Sample (site no.)</th>
<th>TS (no. of pixels)</th>
<th>ES (no. of pixels)</th>
<th>ES (%TS)</th>
</tr>
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<tr>
<td>1 (1)</td>
<td>3,129,176</td>
<td>794,854</td>
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<td>2 (2)</td>
<td>856,017</td>
<td>69,588</td>
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<td>3 (3)</td>
<td>1,383,333</td>
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<td>4 (4)</td>
<td>647,458</td>
<td>188,567</td>
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</tr>
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<td>5 (5)</td>
<td>1,364,564</td>
<td>78,932</td>
<td>5.78</td>
</tr>
<tr>
<td>6 (6,7)</td>
<td>1,313,764</td>
<td>60,080</td>
<td>4.57</td>
</tr>
<tr>
<td>7 (8)</td>
<td>1,574,772</td>
<td>419,829</td>
<td>26.66</td>
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<tr>
<td>8 (9)</td>
<td>1,547,037</td>
<td>335,495</td>
<td>21.87</td>
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<tr>
<td>9 (10)</td>
<td>1,846,370</td>
<td>317,682</td>
<td>17.21</td>
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</table>

TS = total tissue surface; ES = total epithelial rests surface; ES (%TS) = percentage epithelial rests surface out of the total surface.
feature of the samples was their homogeneity. Most of the samples were composed of dense connective tissue. Epithelial remnants were found in all samples in different proportions, ranging from 4.57% to 29.12% (median: 17.21%; mean: 16.36% ± 9.75%). Figures 1 and 2 show clinical and histologic examples.

Discussion

The purpose of this case series was to clinically and histologically evaluate a novel method for deepithelialization of a free CTG, performed before harvesting, using round diamond burs mounted on a low-speed handpiece. The mean root coverage obtained was 89% ± 24.86% (range: 25% to 100%) with complete root coverage in 80% of cases. These results are in agreement with the results presented in a systematic review by Chambrone and Tatakis.

The applied deepithelialization method applied no adverse effect on the esthetic outcome of the root coverage procedures. The recession esthetic scores in the present study ranged between 7 and 9 (mean: 7.44 ± 1.01) for all but one partial failure case (which scored 1) and two adjacent recessions where a keloid-like appearance and partial root coverage was observed (both scored 6).

Various methods of graft deepithelialization retrieved from the lateral palate have been described both extra- or intraorally in the literature, using a blade, a back-action periodontal surgical chisel, dental laser (Er,Cr:YSGG), or a coarse, high-speed bur. In the present case series, the use of the surgical contrangle handpiece with a coarse and then a fine bur allowed the elimination of most of the epithelium, which obtained a smooth, uniform bleeding surface. This approach allows the treating clinician to obtain a superficial graft containing larger amounts of lamina propria (dense fibrous connective tissue) with lower contents of FGT. The presented deepithelialization procedure with use of low-speed diamond burs is gentle and fully controllable. Another advantage of the in situ deepithelialization mode is that it considerably shortens the time in which the graft does not receive any type of nourishment. This deepithelialization method allows the low-discomfort and low-morbidity harvesting of a uniform, dense CTG for the treatment of single and multiple gingival recessions. Furthermore, the donor site may be extended to the distal aspect of the second molar, not limiting it to the premolar to first molar zone.

The histologic evaluation of all samples showed that they were mainly composed of dense connective tissue, and all samples presented with spare epithelial remnants of different proportions (mean: 16.36% ± 9.75%). The presence of epithelium did not seem to interfere with the clinical outcome. These findings are in agreement with those described by Azar et al, where the epithelium was eliminated with a blade, and McLeod et al, where the epithelium was eliminated with a chisel, and both studies still had remnants of epithelium in all of their samples without jeopardizing the results. The fate of the epithelium is not known. In a study by Harris, residual epithelium remained in 24 of 30 CTGs that were used for root coverage procedures. Despite the epithelium remnants, the clinical success was not compromised, resulting in a mean root coverage of 97.9% in 27 patients with Miller Class I or II recession defects. When using a superficial subepithelial CTG for root coverage, the potential complication of these epithelial remnants is the development of cysts. Although some case reports have been described on the formation of a surgical cyst or cyst-like area developing secondary to a SCTG, their presence does not seem to hamper the clinical and esthetic success of the root coverage procedure.

Conclusions

Within the limitations of this case series, it seems that this in situ deepithelialization technique using a bur is simple and valuable, with one minimal morbidity and no postoperative complications. The limited epithelial remnants did not interfere with the clinical and esthetic results. This technique should be evaluated on a larger sample with a long-term follow-up.

Acknowledgments

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References