

## CASE STUDY

# Uncovering of transplanted connective tissue graft: Clinical and histological evaluation

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## Abstract

**Background:** Periodontal plastic surgery aims to restore recessions and dehiscence around teeth and implants. Several techniques, such as subepithelial connective tissue graft (CTG), were proposed with the main outcome of improving volume and root coverage. Nevertheless, this surgery might not improve the keratinized tissue width. Thus, the primary aim of this case report was to describe the possible increase in keratinized tissue after a subepithelial CTG and simultaneously use the previously harvested graft as a source for covering an adjacent tooth.

**Methods:** A 38-year-old patient presented brushing discomfort 2 years after undergoing periodontal plastic surgery with a CTG from the palate to cover a recession. Despite the increased thickness of the soft tissue, brushing discomfort was not reduced because the tissue quality remained unchanged. Therefore, a surgical procedure in the area of teeth 3.2–3.4 was performed to remove the more superficial masticatory mucosa and to induce keratinization of the previously grafted connective tissue.

**Results:** After 6 months, the epithelium appears to be clinically and histologically keratinized, with characteristics comparable to those of the original tissue.

**Conclusions:** The connective tissue grafted maintains the potential to induce keratinization over time, if it is exposed.

## KEYWORDS

connective tissue, histology, keratinized tissue, root coverage, tissue graft

## Key Points

- **Why is this case new information?**  
To the best of our knowledge, this is the first case report in the literature with a histological evaluation in a human of the tissue grafted exposed after 2 years.
- **What are the keys to successful management of this case?**  
The graft covered by the alveolar mucosa did not induce keratinization of the epithelium of the overlying mucosa.
- **What are the primary limitations to success in this case?**  
The main limitation of this study is that it is a singular case report.

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## INTRODUCTION

The oral mucosa is subdivided into the lining mucosa, the masticatory mucosa, and the specialized mucosa.<sup>1</sup> The gum is the part of the chewing mucosa that covers the alveolar process, surrounds the cervical portion of the teeth, and is made up of a layer of connective tissue (lamina propria) and keratinized epithelium. The lining mucosa is the non-keratinized tissue that begins apical to the mucogingival line, covers the basal part of the alveolar process, and continues into the vestibular fornix and floor of the mouth.<sup>2</sup> It has been shown that the characteristics of the gingiva are genetically determined and not the result of an adaptation to environmental stimuli.<sup>3</sup> In fact, it is the connective tissue that determines the overlying epithelial differentiation.<sup>4</sup> Histological studies have shown that the palatal connective tissue does not have the ability to induce the transformation of the epithelium of the alveolar mucosa into keratinized epithelium,<sup>5</sup> which has also been demonstrated by several clinical studies.<sup>6,7</sup> However, the behavior of the connective tissue grafted beneath the lining mucosa after this mucosa has been eliminated has rarely been observed. The aim of this case report was to determine whether the connective tissue harvested from the palate retains the ability to originate keratinized epithelium after it had been submerged in the masticatory mucosa for 2 years.

## MATERIALS AND METHODS

### Patient and clinical case information

A 38-year-old patient, in excellent health, came to our practice for the treatment of gingival recession (Figure 1). The patient presented brushing discomfort, especially at the level of teeth 27 and 22, despite having a history of periodontal plastic surgery with connective tissue grafted from the palate about 2 years earlier (Figure 2A). On clinical observation, we noted voluminous vestibular soft tissue at the level of teeth 21, 22, and 23, and scars could be seen due to a previous surgery (Figure 2A). Presumably,

the patient underwent periodontal plastic surgery with a bilaminar approach: The increase in the thickness of the soft tissue had no effect on brushing discomfort because the quality of the tissue did not improve. The patient's primary objective was to solve the brushing discomfort. She was offered a first intervention in the area of teeth 21–23 to eliminate the superficial masticatory mucosa, which was mobile with respect to the underlying plane and to induce keratinization of the previously grafted connective tissue. A second surgery at the level of tooth 27 would then be carried out to increase the gingival thickness as well as the keratinized gingiva. All risks and benefits involved in the procedures were explained to the patient before she signed an informed consent form. The present study was conducted according to the Declaration of Helsinki and the CAse REports (CARE) case report guidelines.<sup>8</sup> The patient had a good full-mouth bleeding score and full-mouth plaque score, but she was instructed on brushing technique because the generalized gingival recessions and the abrasion she presented at the level of the papillae and necks of the teeth are attributable to an incorrect brushing technique. Carious lesions that were present were also treated.

All surgeries were performed by an expert operator (AF) with the aid of an operating microscope between September 2014 and October 2015.

### First surgery session

A first surgery was planned to uncover the connective tissue previously grafted in the area of teeth 20, 21, and 22 with the aim of inducing keratinization once the masticatory mucosa above it had been removed. The surgical site was anesthetized using mepivacaine–epinephrine 1:100,000 and a 2-mm paramarginal half-thickness horizontal incision as well as two vertical incisions were made with an external bevel inclined 45°. These incisions were connected with a horizontal incision about 10 mm apically. At this point, keeping the 15C scalpel parallel to the tissues, mucosa of approximately 1-mm thick was removed uniformly. Finally, a ball-shaped diamond bur was used to complete the



**FIGURE 1** Baseline intraoral clinical photos.



**FIGURE 2** (A) Baseline clinical extension of the harvested connective tissue graft (CTG) and soft tissue thickness of the treated teeth, (B) removal of the mucosal layer and connective tissue exposition through the use of a scalpel and a diamond bur, and (C) 6 months after CTG exposure there was a gain of Keratinized tissue width.

exposure of the deep connective tissue and smooth the tissue. Six months later, the tissue showed the phenotypic characteristics of palatine fibromucosa (Figure 2B,C).

## Second surgical session

After 6 months, it was decided to treat tooth 27, which presented a 3-mm vestibular recession associated with the loss of interproximal attachment (Figure 3A).<sup>9</sup> The patient also complained of discomfort when brushing, due to the inadequacy of the keratinized tissue, which did not allow her to have optimal plaque control in this area. It is demonstrated that a lack of keratinized tissue width (KTW) was found to be more susceptible to further attachment loss.<sup>10</sup> The absence of keratinized adherent tissue led to the use of a technique that increases the quality and quantity of soft tissue. The envelope technique was performed as described by Langer and Langer,<sup>11</sup> with the aim of leaving the connective graft partially exposed to guarantee that a portion of tissue would become keratinized.<sup>7</sup> A split-thickness flap including the interdental papillae in the flap design was elevated. The vestibular area of tooth 22 was chosen as the donor site for the connective tissue graft (CTG) because the thickness (measured with an endodontic file) was 4 mm. The epithelial-connective tissue from

the donor site was carefully de-epithelialized before being sutured at the level of the enamel–cement junction of tooth 27. The half-thickness flap of the recipient site was then sutured back to its preoperative position with a sling suture, leaving the mid-buccal portion of the graft exposed (Figure 3B–I).

## Tissue collection and histologic analysis

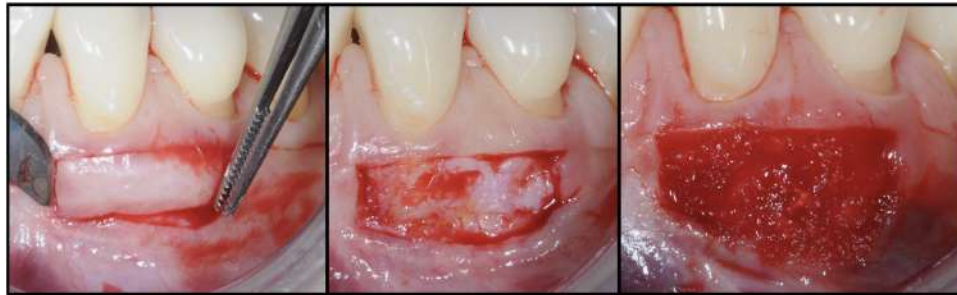
During the second surgical session, two specimens of epithelial-connective tissue (approximately 2 × 2 mm) were collected: a sample of the donor site (vestibular zone 23–21) and a sample of the palatine fibromucosa in zone 14, 3 mm away from the gingival margin (Figure 4). The two biopsy areas were protected with fibrillar collagen.

The biopsies were immersed in fixative solution (4% paraformaldehyde in 0.1 M phosphate-buffered saline [PBS], pH 7.35) and maintained for 24 h at 4°C. Then, they were washed in 0.01 M PBS and stored overnight at 4°C. Finally, they were placed in a 30% sucrose solution in PBS for 3 days at 4°C. The biopsies were frozen for cryostat sectioning and serially cut at a thickness of 25 μm using a Leica CM1900 cryostat. The sections were stained with hematoxylin and eosin using a standard procedure. The





**FIGURE 3** (A) The baseline view of the 4.3 needing a periodontal plastic surgery, (B) flap design, (C) flap elevation, (D,E) CTG harvested from the mandible placement, (F) primary flap repositioning, (G-I) Six months follow up and clinical measurements.



**FIGURE 4** Epithelial CTG harvested from the mandible and the placement of fibrillar collagen (Avitene, Bard Davol) in the donor site.

sections were air-dried, cleared in xylene, and cover slipped with Entellan. The sections were photographed at  $\times 10$ ,  $\times 20$ , and  $\times 40$  magnification using a professional microscope connected to a digital video camera. All images obtained were processed with Adobe Photoshop CS4.

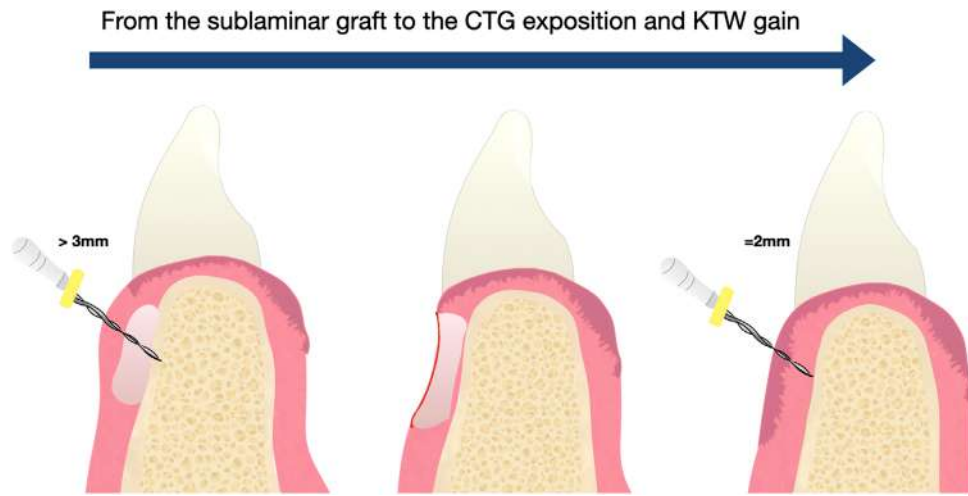
## RESULTS

The postoperative healings were uneventful. Clinically, after 6 months, it was possible to notice an increase in keratinized tissue ( $4 \times 12$  mm) of the area from which the chewing mucosa overlying the connective tissue previously taken from the palate had been removed. The thickness, measured with an endodontic file, was about 4 mm; it allowed us to sample about 1.5 mm of epithelial-connective tissue (Figure 5). Six months after collection, the area was com-

pletely re-epithelialized, adhered to the underlying tissues and of a reduced thickness (Figure 6). The patient declared that she no longer had any discomfort with brushing maneuvers.

After 21 days, the area of the palatal biopsy had healed completely. The tissue at the grafted area appeared clinically free of inflammation and tightly adapted to the tooth. The thickness was increased considerably, and the keratinized tissue appeared to have increased by approximately 3 mm. The gingival recession was covered partially, and the patient was satisfied because she no longer had brushing discomfort (Figure 3G–I).

Histological analysis revealed no qualitative or quantitative differences between the tissue samples taken from the palate and the donor site. Observing the samples at  $\times 10$ ,  $\times 20$ , and  $\times 40$  magnification, the tissues seem to have the same histological characteristics: keratinized, stratified,



**FIGURE 5** Representation of the measurement at baseline and 6 months after the CTG exposition and the associated gain of KTW. KTW, keratinized tissue width.



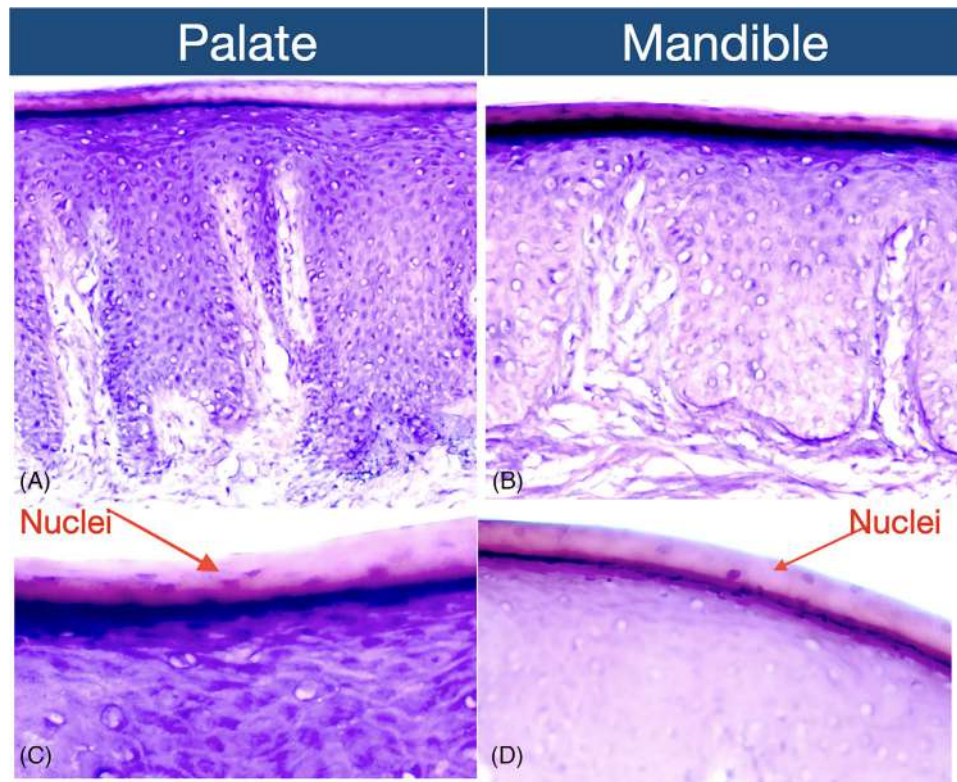
**FIGURE 6** Timetable, clinical and histological results.

squamous epithelium (Figure 7). It is possible to appreciate the different layers from the deepest to the superficial: the stratum basale, the stratum spinosum, the stratum granulosum and the stratum corneum. It should be noted that the cells of the stratum corneum in these sections contain remnants of the nuclei; in this case, the epithelium is denoted as parakeratinized.

### DISCUSSION

Augmentation of keratinized tissue around teeth with mucogingival defects has always been a goal for periodontists. Indeed, a certain amount of keratinized tissue has been considered necessary for the maintenance of gingival health and to prevent gingival recession.<sup>12,13</sup> Therefore,





**FIGURE 7** Histological analysis in hematoxylin-eosin of two samples collected from the palate and mandible showing at low and high magnification (10X; A-B) and (40X; C-D) similar aspect and the presence of nuclei in the stratum corneum.

increasing the width of the attached gingiva in patients who feel discomfort during cleaning is advisable to prevent further loss of attachment.<sup>10</sup> The keratinized adherent gingiva is separated from the non-keratinized alveolar mucosa by the mucogingival junction and are distinguished histologically by some peculiarities such as the characteristics of collagen fibers, the connective papillae, and the presence or absence of a keratin layer on the surface of the epithelium.

In the 1960s and 1970s, investigations of soft tissue grafts showed that it was possible to augment keratinized tissue through free gingival grafting,<sup>14</sup> as well as subepithelial connective tissue grafting.<sup>15</sup> In current practice, subepithelial CTGs are used widely in periodontal and peri-implant plastic surgery to augment the soft tissue volume, to ensure an adequate width of keratinized tissue, to correct mucogingival problems, and to improve the aesthetics at teeth as well as dental implant sites.<sup>16</sup> In the case of aesthetic problems associated with gingival recessions, the gold standard is CTG positioned beneath coronally advanced flaps (CAF) to obtain both root coverage and improved KTW (1 mm gain).<sup>17</sup> However, when a significant amount of keratinized tissue is desired, other surgical techniques must be applied. The regeneration of keratinized attached gingiva appears to be influenced by the tissue phenotype of the subepithelial connective tissue. It has been shown in an animal model that connective tissue transplanted from the masticatory mucosa into a bed

receiving the lining mucosa induces the formation of lining epithelium with characteristics of that of the donor site.<sup>4</sup> Bernimoulin and Schroeder<sup>18</sup> also demonstrated in humans that connective tissue of the lamina propria is mainly responsible for the phenotypic characteristics of the overlying epithelium.

The results of the histological studies are consistent with clinical data from various studies. Borghetti and Louise,<sup>19</sup> in a controlled clinical study in which CTGs were covered by a double papilla flap, demonstrated that the position of the mucogingival junction was stable over time. This indicates that only the portion of the CTG covered by the keratinized connective tissue produced keratinized tissue after surgery, while the part of the graft located beyond the mucogingival junction and covered by the alveolar mucosa did not induce keratinization of the epithelium of the overlying mucosa. Further exploration of this topic was conducted by Di Domenico et al. (2023), in a prospective study that focuses on the increase of keratinized tissue obtained following multiple CAF (M-CAF) with selective use of CTG.<sup>20</sup> Based on a clinical and histological analysis, the authors conclude that the connective graft cannot induce any modifications in the overlying epithelium if it remains completely submerged by the flap overlying it; on the other hand, if the graft remains exposed because of apical position of flap, the amount of keratinized tissue may increase. In clinical terms, the sites treated with MCAF + CTG obtain

the greatest increase in keratinized tissue in the event of flap recession and connective tissue exposure.<sup>20</sup>

Another clinical study comparing an envelope flap + CTG to CAF + CTG demonstrated that a greater increase in the width of keratinized tissue could be expected in sites treated with the envelope flap technique, compared with CAF, as part of the grafted connective tissue remains exposed during healing.<sup>7</sup> The increase in keratinized tissue at sites treated with CAF + CTG in the aforementioned study can be attributed to a possible shift in the overlying flap in an apical direction during wound healing, thus exposing the CTG. The connective tissue grafted maintains the potential to induce keratinization over the time if it is uncovered.

## CONCLUSION

This case report is in line with the studies cited above and demonstrates that 6 months after uncovering the CTG taken from the palate and positioned under the masticatory mucosa, the epithelium appears to be clinically and histologically keratinized, with characteristics that are comparable to those of the original tissue. Further clinical and histological studies with a larger number of patients are necessary to confirm the current results and evaluate the type of healing obtained from a histological point of view.

## AUTHOR CONTRIBUTIONS

**Adriano Fratini:** Conceptualization (lead); writing—original draft (lead); formal analysis (lead); writing—review and editing (equal); resources (lead). **Leonardo Mancini:** Writing—review and editing (equal). **Margherita Giorgia Liguori:** Writing—original draft preparation; visualization (equal); validation (equal). **Stefano Gotti:** Writing—original draft; preparation; investigation (equal). **Enrico Marchetti:** Data curation (equal); investigation (equal); methodology (equal).

## CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

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