

## CASE REPORTS

# Subepithelial connective tissue graft for root coverage: clinical case reports and histologic evaluation

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### Abstract

If the clinical goal of the periodontal plastic surgery is the complete root coverage of gingival recessions, then the microscopically goal standard is to obtain a true periodontal regeneration. Only the histological examination can reveal cellular events unrolled into the grafted tissue-root surface interface and the nature of the clinically observed attachment. To our knowledge, there are no studies that obtained the biopsies of grafted tissues from humans, *in vivo*, and so, the aim of the present study was to develop such a method, using a bone marrow biopsy needle. Two patients with gingival recessions were treated with coronally advanced flap and subepithelial connective tissue graft, and the clinical and histological results were observed. Complete root coverage was obtained for both cases. Clinically, the grafted tissues seemed to be attached to the root surfaces. The histological findings revealed dense grafted tissues, providing long-term stability of the area. No ligament or bone, characteristic for periodontal regeneration were observed. However, no sign of healing characterized by a long junctional epithelium was observed either. This indicated that the healing that occurred in our cases was a long connective tissue attachment, which has been shown to be stable over time. However, even if the method we proposed is not invasive, it is very difficult to convince the patient to accept a biopsy elevation once the exposed root surface was covered.

**Keywords:** gingival recession, surgery, fibroblasts, connective tissue.

### Introduction

Esthetics is becoming an important concern in dentistry. Harmonious and symmetric alignment of the teeth with a consistent shape, size and color is essential, and the harmonious gingival morphology has been emphasized. Gingival recession is one of the soft tissue problems faced by dentists and patients. It is frequently associated with esthetic concerns, fear of tooth loss and root hypersensitivity. There are several approaches for treating gingival recession: flap surgical procedures and procedures using graft materials in combination with flap operations. Many studies have confirmed that Miller Class I and II recessions, for which the level of periodontal proximal tissues is not affected, can be predictably covered by various periodontal plastic surgical procedures, including pedicle flaps, subepithelial connective tissue grafts (CG), acellular dermal matrix grafts, and guided tissue regeneration [1–5].

If the clinical goal of the periodontal plastic surgery is the complete root coverage, then the microscopically goal standard is to obtain a true periodontal regeneration. Only the histological examination can reveal cellular events unrolled into the grafted tissue-root surface interface and the nature of the clinically

observed attachment. However, the nature of the attachment between the grafted tissue and the root surface is not well understood and in clinical practice, only the clinical success is measured.

In spite of predictable clinical outcomes with the use of coronally advanced flap (CAF) and CG, its healing process and histological outcome still remain controversial, because for obvious ethical reasons studies evaluating human histology after the use of these techniques are scarce. The histologic evidence derived from animal studies or from studies which realized the biopsies after the extraction of the concerned teeth for various reasons. CAF and CG were associated to some degree of periodontal regeneration [6–8]. Other results showed that healing occurs primarily by the formation of a long junctional epithelium or a connective tissue adhesion interface between the root surface and the graft [9, 10]. To our knowledge, there are no studies that obtained the biopsies of grafted tissue from humans, *in vivo*. The aim of the present study was to develop a method to study the healing process after gingival grafting for which the teeth must not be extracted and to observe the histologic results after treatment of gingival recessions with CAF and CG, in two patients.

## ☐ Patients, Methods and Results

### Case No. 1

#### Patient and site description

A 35-year-old man visited the Department of Periodontology, "Iuliu Hațieganu" University of Medicine and Pharmacy, Cluj-Napoca, on May 3<sup>rd</sup>, 2007, with a chief complaint of hypersensitivity, fear of tooth loss and gingival recession on the left maxillary canine. The patient was in good general health and was a non-smoker.

An intraoral examination revealed a good oral hygiene status (full-moth plaque score 22%) [11]. A 5 mm high-Class I Miller recession on the maxillary left canine, with the lack of attached gingiva, but the presence of a narrow band of keratinized tissue were observed (Figure 1).



**Figure 1 – Case no. 1.**  
*A 5 mm high-Class I Miller recession on the maxillary left canine.*

The clinical findings are shown in Table 1.

**Table 1 – Case no. 1. Clinical parameters at baseline and at 12 months follow-up visit**

Measured parameters on buccal surface	Baseline [mm]	12 months follow-up [mm]
Recession high: Middle	5	0
Probing depth: Mesial	2	2
Middle	2	2
Distal	2	2
Keratinized gingiva: Middle	2	5

Treatment plan was made for root coverage. Initial therapy, including scaling and oral hygiene instruction with proper tooth brushing method, were performed.

#### Experimental design

The surgical and histological protocols are part of a research that compares the outcomes of some surgical root coverage techniques; the protocols were approved by the Ethical Committee of the University. Informed consent was obtained from the patient after careful explanation of the surgical procedure, prognosis, and possible complications.

The surgical procedures were performed by the same surgeon (AR), who was not involved with the clinical measurements or sample elevation. The clinical measurements were made by two experienced and calibrated investigators (AS, RC) and the sample was elevated by another investigator (HG). A single experienced examiner (ID) was responsible for the histological examination.

#### Surgical procedure

The design of CAF was similar to that described by Langer B and Langer L (1985) [12]. After local anesthesia

of the recipient with 2% articaine with epinephrine 1:100 000 (Septanest<sup>®</sup>, Septodont), an intracrevicular incision was made through the bottom of the crevice, and a partial thickness flap was elevated. Two vertical releasing incisions were made without including the papillae adjacent to the area of the gingival recession. The flap was divided beyond the mucogingival junction and was extended until it could be passively positioned coronally over the defect without tension. The exposed root surface was planed with a 5/6 Gracey curet. The CG was removed from the palate, using a single incision technique [13]. The graft was timed and sutured over the defect with a 5–0 bioabsorbable sutures (Vicryl<sup>®</sup>, Johnson & Johnson), and the previously reflected partial-thickness flap was coronally positioned to cover the entire graft. The flap was sutured in place with sling sutures (5–0 bioabsorbable). The patient was instructed to discontinue tooth brushing for 14 days and to avoid trauma at the surgical site. A 0.2% Chlorhexidine digluconate rinses (Corsodyl<sup>®</sup>, GlaxoSmithKline) was prescribed two times a day. The sutures were removed after 14 days. The patients were recalled for prophylaxis at three month postoperatively. The clinical measurements were recorded at baseline and at 12 months post-operative visit.

#### Biopsy preparation

At 12 months follow-up visit, a biopsy was performed under local anesthesia. The tissue was elevated from the attached gingiva, at the previous level of gingival margin (5 mm apically from the post-operative gingival margin), using a Bone Marrow Biopsy Needle ET<sup>®</sup> 8/10 (Bloodline). The entire width of the tissue above the root surface was elevated.

The biopsy specimen was fixed in 10% neutral buffered formalin solution at room temperature for one day. Following fixation, the biopsy was processed in Leica ASP 300<sup>®</sup> (Leica) Tissue Processor (Formalin 1 30 minutes, Formalin 2 30 minutes, Ethanol 70% one hour, Ethanol 80% 1.30 hours, Ethanol 96% 1.3 hours, Ethanol absolute one hour, Ethanol absolute 1.30 hours, Ethanol absolute 1.30 hours, Xylene 1 1.30 hours, Xylene 2 1.30 hours, Paraffin 1 one hour, Paraffin 2 two hours, Paraffin 3 three hours). The sample was embedded in paraffin and 4 µm-thick sections were obtained from the specimen with Leica RM2145 Microtome<sup>®</sup> (Leica). The sections were stained with Hematoxylin and Eosin in Leica Autostainer XL<sup>®</sup> (Leica). Masson's trichrome stain was performed for evaluation of the type and amount of extracellular collagen and Orcein stain for evaluation of elastin fibers.

The slides were examined and photographed using Zeiss Axioscope<sup>®</sup> 2 plus light microscope connected to a Sony DSC–S85 digital camera at ×4, ×10, ×20, ×40 and ×100 magnifications. The measurements were determined on light microscope with Carl Zeiss Object-mikrometer<sup>®</sup> 5+100/100 mm.

#### Clinical observations

Wound healing was uneventful. The defect was 100% covered after 12 months (Figure 2).

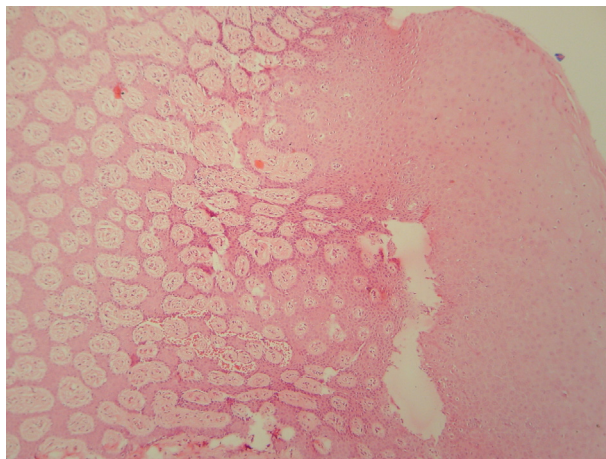
**Figure 2 – Case no. 1.**  
*View of treated area 12 months after root coverage surgery.*



The amount of keratinized tissue was 5 mm and there was no difference in the probing depth before and after surgery (Table 1). The integration of the grafted tissue in the surrounding tissues was recorded.

#### **Histological observations**

The epithelium was slightly thickened (1 mm) and parakeratinized. Rete ridges were elongated, projected and anastomosed into the gingival connective tissue. The connective tissue subjacent to the epithelium was composed of collagen fibers of varying thicknesses, with fusiform cells and rare blood vessels. Inflammatory infiltrate was absent. No bone or osteoid were observed (Figure 3).



**Figure 3 – Case no. 1. Histologic view of the biopsy:**  
*rete ridges are elongated and anastomosed into the gingival connective tissue (HE stain, ×100).*

### **Case No. 2**

#### **Patient description**

A 39-year-old man visited the Department of Periodontology, “Iuliu Hațieganu” University of Medicine and Pharmacy, Cluj-Napoca, on September 10<sup>th</sup>, 2008, with a chief complaint of fear of tooth loss and gingival recessions on the right maxillary canine and first premolar. The patient was in good general health and was a non-smoker. Oral hygiene status was acceptable (full-mouth plaque score 28%) [11]. A 4 mm high-Class I Miller recession on the maxillary right canine, with 5 mm of attached gingiva, and a 4 mm high-Class I Miller recession on the maxillary right first premolar, with 4 mm attached gingiva were observed (Figure 4).

Cervical erosion lesions were present. The clinical findings at baseline are shown in Table 2. The patient was scheduled to undergo root coverage.



**Figure 4 – Case no. 2.**  
*A 4 mm high-Class I Miller recession on the maxillary right canine and a 4 mm high-Class I Miller recession on the maxillary right first premolar.*

**Table 2 – Case no. 2. Clinical parameters at baseline and at 12 months follow-up visit**

Measured parameters on buccal surface	Maxillary right canine		Maxillary right first premolar	
	Baseline [mm]	12 months follow-up [mm]	Baseline [mm]	12 months follow-up [mm]
Recession high: Middle	4	0	4	0
Probing depth: Mesial	1	1	2	2
Middle	1	1	2	2
Distal	2	2	2	2
Keratinized gingival: Middle	5	8	4	7

#### **Experimental design**

The experimental design was the same as for the first case, although the area requiring root coverage in this case was greater than in case no. 1.

#### **Clinical observations**

The grafted area healed well and full coverage (100%) was achieved for both recessions, at 12 months follow-up visit (Figure 5).

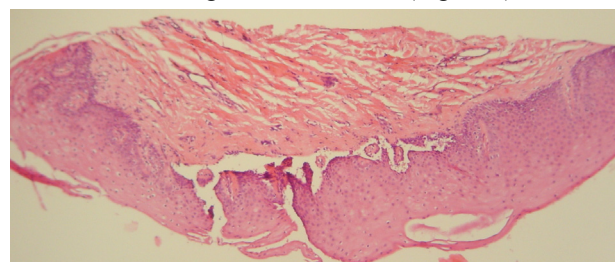


**Figure 5 – Case no. 2.**  
*View of treated area 12 months after root coverage surgery.*

There was a 3 mm gain in keratinized tissue in both areas and there was no change in probing depth compared to before surgery (Table 2). The general color and volume match were successful.

#### **Histological observations**

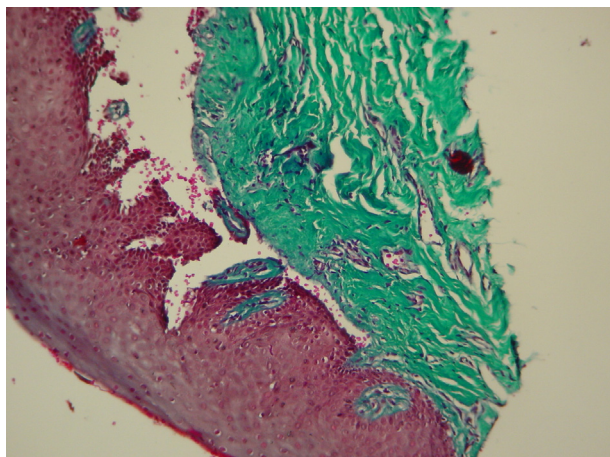
A thin (0.4 mm), parakeratinized epithelium was observed. Rete ridges were flattened (Figure 6).



**Figure 6 – Case no. 2. Histological view of the biopsy:**  
*flattened rete ridges are observed (HE stain, ×40).*

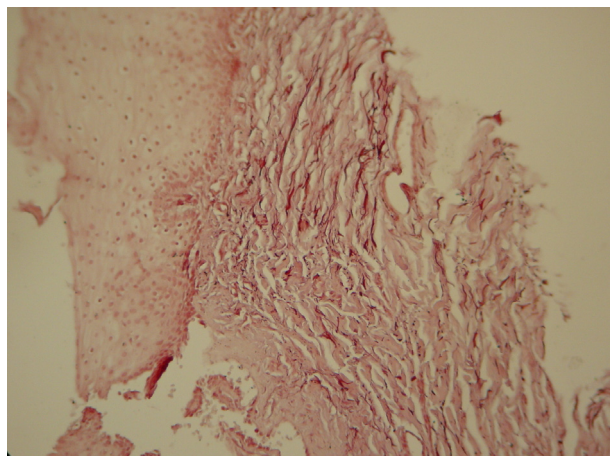


The connective tissue subjacent the epithelium was thick, composed of large bundles of collagen with fusiform cells and rare blood vessels (Figure 7).



**Figure 7 – Case no. 2. Histological view of the biopsy: a thick connective tissue subjacent the epithelium is present (Masson's trichrome stain,  $\times 100$ ).**

No inflammatory infiltrate, bone or osteoid were observed. Few elastin fibers were present on the sample stained with Orcein (Figure 8).



**Figure 8 – Case no. 2. Histological view of the biopsy: few elastin fibers are present (Orcein stain,  $\times 100$ ).**

## Discussion

The clinical and patient centered outcomes were excellent. Complete root coverage was obtained for both cases. No scars resulting in esthetically displeasing appearance were observed. Clinically, the grafted tissues seemed to be attached to the root surfaces. The obtained clinical outcomes are not surprising having in view that CAF with CG may be still considered the gold standard procedure for covering Miller Class I and II gingival recessions [4]. One of the advantages of CG with a CAF over others procedures is that it produces a larger increase in the keratinized tissue compared with repositioned flaps alone [14, 15], as the results associated with the presented cases revealed. The presence of thick attached keratinized tissue may constitute a protective factor against marginal inflammation or trauma.

The histological findings revealed dense grafted tissues, providing long-term stability of the area. The rete ridges hyperplasia observed for the first case provides

mechanical resistance to external irritations. No ligament or bone, characteristic for periodontal regeneration were observed. However, no sign of healing characterized by a long junctional epithelium was observed either. This indicated that the healing that occurred in our cases was a long connective tissue attachment, which has been shown to be stable over time [7]. No periodontal regeneration associated with CAF and CG was observed by other studies either. The healing process was characterized by a long junctional epithelium [9, 10].

Some studies has shown that the pedicle graft with or without CG may heal by periodontal regeneration. Histological examination of a laterally positioned flap has shown new connective tissue attachment [16]. New connective tissue attachment (3.9 mm), including periodontal ligament was observed to be associated with a CAF with CG [6]. The histological evaluation of a CG revealed a healing process characterized by 1 mm new bone formation, new periodontal fibers, new cementum, and new connective tissue attachment (3–5 mm) [7].

CG and CAF plus Emdogain® (Enamel Matrix Derivative) were associated to the periodontal regeneration (1.87 mm of new bone, and 2.25 mm connective tissue anchored in 0.06 mm new cementum) [8]. A CG under a CAF was associated to the partial root coverage and long junctional epithelium, with a minimal new attachment and bone formation [17]. Regeneration is clearly possible with other periodontal plastic surgery procedures, such as free gingival graft, Acellular Dermal Matrix Graft and guided tissue regeneration [3].

## Conclusions

The concern with the nature of the grafted tissue attachment is based on the concept that the ultimate goal of periodontal treatment is to fully restore the attachment apparatus. Current available therapies have shown limited and rather unpredictable results. Even if the histological observations associated with the presented two cases failed to observe a periodontal regeneration, the nature of connective tissue attachment seemed to be stable in time. We have in view other similar cases, in order to obtain more evidence. However, even if the method we proposed is not invasive, it is very difficult to convince the patient to accept a biopsy elevation once the exposed root surface was covered.

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