Histology of Connective Tissue Graft. A Case Report

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Background: *Few investigations can be found in the literature on the histological nature of the attachment of connective tissue grafts to root surfaces previously exposed by recession.*

Methods: *In this case report, a 24-year-old patient was treated with a connective tissue graft combined with a partial-thickness coronally positioned flap for root coverage of Class I Miller recessions at the maxillary right and left canines and first premolars. The treated sites exhibited 83% and 100% root coverage on the right and left sides, respectively. Twelve months later, the case required extraction of all 4 first premolars for orthodontic reasons. Two conservative block sections including the maxillary first premolars with the buccal soft tissues were obtained and processed histologically in a bucco-palatal plane.*

Results: *Histological analysis showed that healing occurred via a long junctional epithelium throughout the major portion of the previous recession site. Only minimal signs of new cementum-like tissue formation could be seen in the apical portion of the recession area coronal to the base of the instrumented root surface. No root resorption or ankylosis could be detected in any of the serial sections.*

Conclusions: *The findings of this case report outline the possible variations in the histological outcome of connective tissue grafts. These variations can be attributed to differences in size and shape of the recession defects and flap positioning at the end of surgery.*
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KEY WORDS

Mucogingival surgery; grafts, subepithelial; grafts, connective tissue; gingival recession/surgery; tooth root; surgical flaps; epithelial attachment/ histology.

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Bilaminar techniques have been proposed to enhance the predictability of gingival grafting procedures by improving the blood supply to the grafted tissues.[1-3] The predictability of subepithelial connective tissue grafts and the mucogingival changes associated with these procedures following treatment of recession defects have been documented in several studies.[4-13] In these techniques, a free connective tissue graft is positioned over the recession site and covered by a pedicle flap. Variations include the use of various primary flaps and techniques for harvesting the connective tissue graft.[14]

Although the esthetic and functional success of these techniques has been shown to be predictable,[4-13] the histological aspect of the root coverage has remained in question. Pasquinelli[15] reported 4.4 mm of new connective tissue attachment and 4.0 mm of new bone formation in a 6 mm mandibular premolar recession treated with a free connective tissue graft in a 40-year-old woman. Similar histological findings were described later by Weng et al.[16] who compared the outcome of free connective tissue grafts and guided tissue regeneration using expanded polytetrafluoroethylene (ePTFE) membranes in the treatment of buccal recessions in the maxillary canines of 7 beagle dogs. The authors reported 5.5 mm of new connective tissue attachment formation, amounting to 57.0% of the total coverage height, in the recessions treated with free connective tissue grafts consisting of deep palatal connective tissue and periosteum. No statistically significant differences in the amount of new connective tissue attachment were found between connective tissue-treated sites and recessions treated with ePTFE membranes. More recently, Bruno and Bowers[17] reported the histological results of a human biopsy 1 year following the placement of a subepithelial connective tissue graft. The authors demonstrated that the apical portion of the exposed root surface healed by regeneration (new bone, cementum, and periodontal ligament), while the greatest area of the recession was covered by a connective tissue adhesion.

This paper presents a human case report detailing the histological nature of the attachment of 2 con-

nective tissue grafts to root surfaces previously exposed by recession.

MATERIALS AND METHODS

Patient and Root Coverage Procedure

A 24-year-old woman, a non-smoker and in good health, was referred for root coverage of Class I Miller[18] recessions at the maxillary right and left canines and first premolars. The patient had esthetic concerns and reported a progressively increasing generalized recession pattern, more severe in the listed teeth, despite the correction of her toothbrushing habits and satisfactory levels of plaque control. It was decided to attempt root coverage using subepithelial connective tissue grafts. The preoperative clinical parameters including recession height, width of keratinized tissue, and probing depth were assessed at the deepest point of the buccal recession sites immediately prior to surgery (Table 1). The measurements were recorded to the nearest 0.5 mm using a periodontal probe.‖

A trapezoidal split-thickness flap was elevated at the buccal aspect of the teeth with vertical releasing incisions at the mesial and distal extremities of the horizontal incisions extending beyond the mucogingival junction. The exposed root surfaces were thoroughly planed with curets and back-action chisels up to the marginal level of the crestal bone to reduce root convexity. Following preparation of the recipient bed, a 1 to 1.5 mm thick connective tissue graft was harvested from the premolar/molar region of the palate using 2 parallel horizontal incisions starting about 2 mm apical to the gingival margin. A partial-thickness mucosal flap was prepared without releasing incisions, and the underlying connective tissue was separated from the surrounding connective tissue by making incisions to bone at the mesial, distal, and medial sides of the graft. The graft was then detached from the bony surface using a periosteal elevator. The epithelial marginal collar was removed and the graft inserted into the recipient site. The covering flap was coronally positioned without tension and sutured with 5-0 monofilament sutures leaving about 0.5 mm of the mid-buccal portion of the graft exposed. Sutures were removed 2 weeks postsurgery. The patient was instructed not to brush teeth in the treated sites and to rinse 3 times daily with a 0.12% chlorhexidine rinse for 4 weeks. Mechanical plaque control in the surgical areas was reinstituted at this point. The patient was recalled for prophylaxis every 2 weeks for the first 3 months and once every 4 months afterwards.

Table 1.

Clinical Measurements at Baseline and 11 Months Following Grafting Procedures

	Baseline (mm)			11 Months (mm)		
	PD	Recession	KT	PD	Recession	KT
Right maxillary first premolar	1.0	3.0	0.5	1.0	0.5	3.0
Left maxillary first premolar	1.0	2.5	1.5	1.0	0	5.0

PD = probing depth.
KT = width of keratinized tissue.

Biopsy Procedure

At the recall visit 11 months following the surgical procedures, complete coverage was evident at the left canine and first premolar, while the contralateral sites exhibited root coverage to within 0.5 mm of the cemento-enamel junction (CEJ) (83%) (Table 1, Figs. 1 and 2). One month later, the patient decided to undergo orthodontic therapy requiring extraction of the 4 first premolars. The patient agreed to have both maxillary first premolars extracted along with the coronal portion of the facial tissues. The patient's informed consent was obtained. Two block sections were removed before orthodontic therapy was begun (Fig. 3). The biopsy procedure was carried out as follows: vertical incisions were made at the mesial and distal line angles of the 2 first premolars down to the root surface. The vertical incisions extended approximately 6 mm apically from the gingival margin and were connected at their apical end by a horizontal incision made through the bone and the root. The teeth were then atraumatically extracted along with the facial tissues.

Histologic Preparation

The 2 specimens were immediately rinsed with sterile saline and fixed in 10% neutral buffered formalin solution for histological processing. After fixation, the specimens were decalcified in 5% formic acid for about 4 weeks, embedded in paraffin, and sectioned parallel to the long axis of the tooth in a bucco-lingual direction at a thickness of 5 to 6 µm. The most mid-facial sections were stained with hematoxylin-eosin and analyzed histologically and histomorphometrically.

‖ XP23/UNC15, Hu-Friedy, Leimen, Germany.

Figure 1.
A. *Preoperative view of the right canine/premolar area. The maxillary first premolar exhibits 3 mm of recession with a mid-buccal probing depth of 1 mm and 0.5 mm of keratinized tissue.* ***B.*** *Postoperative results 11 months following the connective tissue graft procedure.*

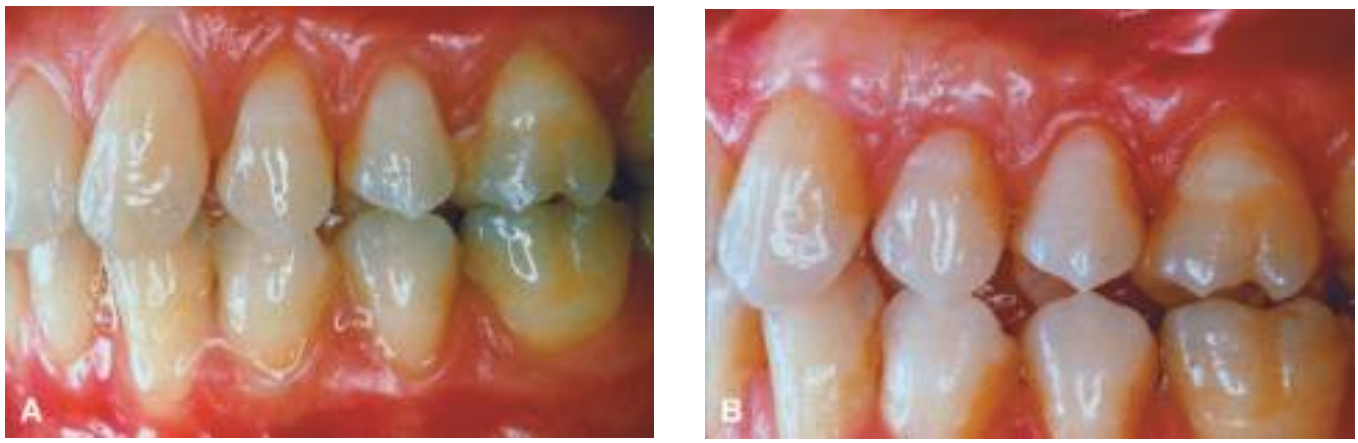

Figure 2.
A. *Preoperative 2.5 mm recession at the buccal aspect of the maxillary left first premolar.* ***B.*** *Eleven-month postoperative root coverage.*

RESULTS

Histologically, the major portion of the exposed root surface in both specimens was covered by a long junctional epithelium totally located on exposed dentin (Fig. 4A). The root surface previously planed was clearly identifiable with the absence of any cementum. Starting from the middle third of the previously exposed recession site, the junctional epithelium was limited to 1 to 2 cells in thickness (Figs. 4B and 4C). In both biopsies, the junctional epithelium ended just coronally to the bone crest. An irregular mineralized tissue formation, mostly acellular in nature, was present at the bottom of both defects in continuity with the old cementum coronally to the apical limit of root instrumentation (Fig. 4D). An artifactual separation was present between this layer of newly formed cementum-like tissue and the dentin. In one specimen, the junctional epithelium had migrated to the coronal limit of the newly formed hard tissue. In the second specimen, the epithelium terminated slightly coronal to the cementum-like formation. In the intervening root area, the dentin surface was covered by a connective tissue arranged parallel to the root surface, suggesting connective tissue adhesion (Fig. 4D).

A clear junction between the graft and the recipient bed connective tissue was not detected in any of the photomicrographs. Residues of the original grafted tissue were identified in an isolated area of loose connective tissue rich in vascular network and populated

Figure 3.
Block specimen including the maxillary left first premolar with the buccal tissues 12 months following root coverage procedure.

by numerous adipocytes laterally to the alveolar crest (Fig. 4A).

Coronal growth of alveolar bone occurred to a minimal extent in both specimens and did not parallel the height of the newly formed cementum-like tissue (Fig. 4D). The alveolar crest was characterized by a high cellular content, woven type of bone, and areas of bone remodeling. No root resorption or ankylosis could be detected in any of the serial sections.

Histomorphometric measurements are reported in Table 2. The postoperative distance between the free gingival margin and the apical extent of the junctional epithelium was 3.80 mm and 3.40 mm in the right and left premolars, respectively. Newly formed bone was found 0.12 mm and 0.21 mm coronally to the apical limit of root instrumentation in the right and left specimens, respectively. The most coronal evidence of new cementum-like tissue was found at 0.38 mm and 0.42 mm from the apical extension of root planing in the right and left teeth, respectively.

DISCUSSION

This human histologic case report demonstrated successful clinical coverage of 2 recession-type defects by connective tissue graft combined with a coronally positioned flap, but a histologic healing through a long junctional epithelium and virtually no regeneration at the bottom of either defect.

Early studies suggested that root coverage of recession defects using pedicle flaps results in a long junctional epithelial attachment.[19-22] Gottlow et al.[21] in 1986 examined the healing of surgically created gingival recessions following treatment with coronally displaced flaps in a beagle dog model. The authors showed that the amount of new attachment formed coronal to the notch prepared in the root at the bone crest varied considerably from one root to another and ranged between 0.9 mm and 4.2 mm. The authors attributed these variations to the method used to assess the amount of regenerated tissue and variations in size and shape of the defects.

When the histologic outcome of free soft tissue grafts is considered in the treatment of gingival recessions, human case reports and animal studies[15-17,23] suggest that healing occurs through regeneration including new bone, new cementum, and new connective tissue attachment. The results of this study indicated a completely different histologic healing with mainly a long junctional epithelium. These differences can be attributed to various factors such as variations in size and shape of the recessions as mentioned above.[21] The histologic outcome in the present report compares with the results of Harris[24] who showed no evidence of regeneration 6 months postoperatively in 2 recessions with a depth of 2 mm and 3 mm successfully treated with connective tissue graft combined with a partial-thickness double pedicle graft. In a second report, the same author demonstrated new bone, new cementum, and connective tissue attachment coronally to the presumed location of the gingival margin at 5 months postoperatively in a 4 mm deep recession.[23] The author suggested that the difference in defect size in the 2 case reports (2 and 3 mm[24] versus 4 mm[23]) could have affected the regenerative potential in the recession sites, with the greater recession depth possibly providing a greater opportunity for regeneration. This hypothesis could seem plausible when considering that all investigations reporting healing of recession sites through regeneration of new cementum, new bone, and new connective tissue attachment[15-17] involved recession depths >5 mm.

Another factor that could explain the intra- or interindividual response can be flap positioning at the end of surgery.[21,25] The use of a coronally displaced flap combined with the use of a connective tissue graft allows for complete coverage of the graft devoid

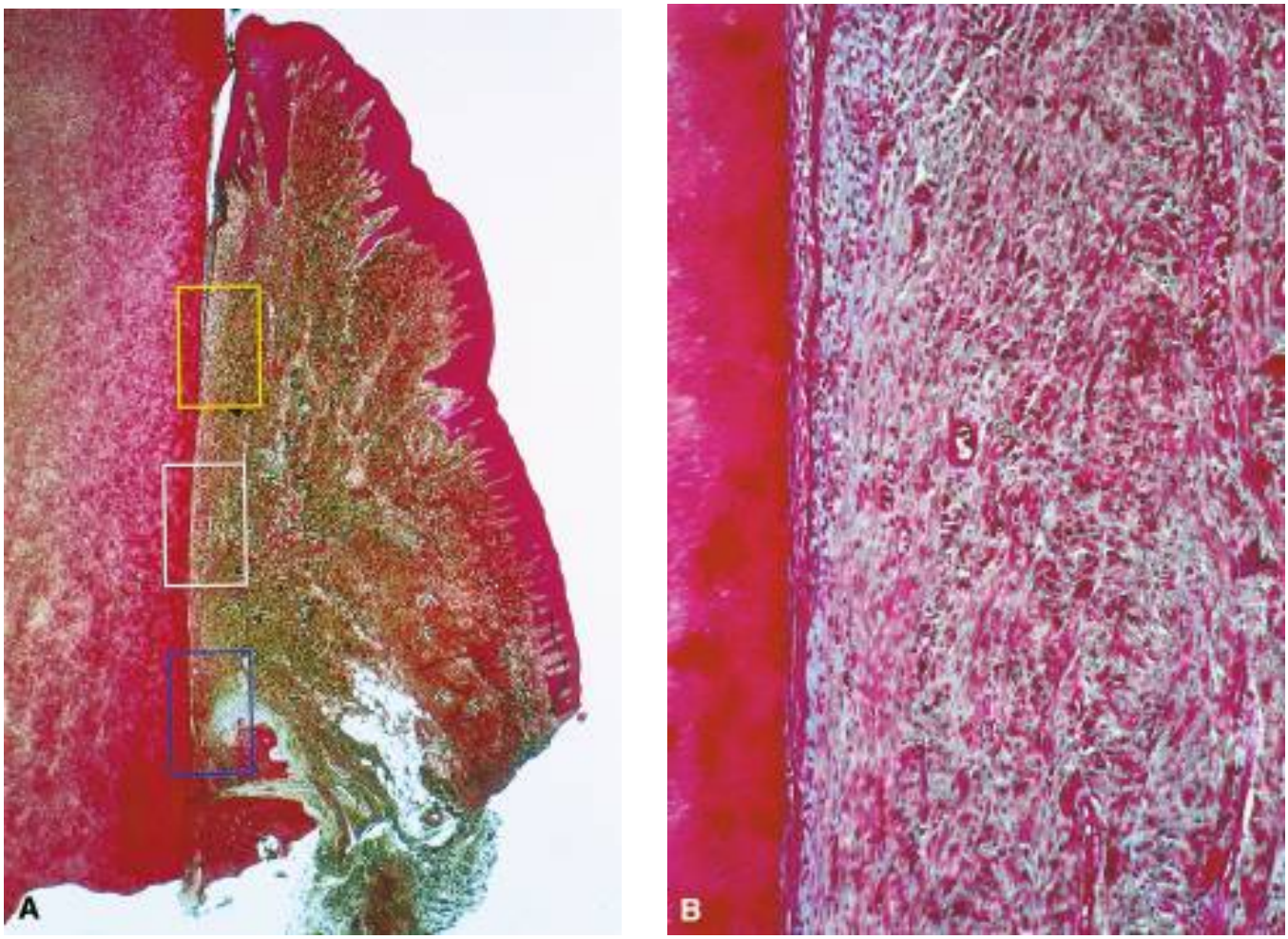

Figure 4.
A. *Low magnification of a mid-facial section of the right first premolar. Note the extension of the long junctional epithelium on the exposed root surface and the presence of adipose tissue in a highly vascularized connective tissue matrix laterally to the alveolar crest. The areas enclosed in the colored frames are shown in greater magnification in Figures* 4B, 4C, *and* 4D *(original magnification ×6).* ***B.*** *Higher magnification of the area within the yellow frame in A. The junctional epithelium is thinning out and transforming into a 1- to 2-cell layer (original magnification ×40).*

of an epithelial collar by the primary flap.[14] In the inlay technique, a split-thickness flap including the interdental papillae in the flap design is elevated; a connective tissue graft with or without an epithelial marginal collar is inserted into the recipient site; and the covering flap is sutured back to its preoperative position, leaving the mid-buccal portion of the graft exposed.[14] These 2 techniques of subepithelial connective tissue graft differ significantly relative to the distance that oral epithelial cells have to migrate in order to reach the coronally proliferating periodontal ligament. Histologically, it might be expected that coronal positioning and adaptation of the flap to the root surface to cover the connective tissue graft determine an early contact between the oral epithelium and the root surface. In addition, the epithelium and connective tissue of the pedicle flap are not subjected to major changes because of the preservation of the blood supply. The epithelial integrity and premature contact of the epithelium with the root surface might allow a faster epithelial downgrowth along the root surface and decrease the time interval during which periodontal ligament cells can form new attachment.[26] In a similar recession defect, the donor tissue in the inlay variant of the connective tissue graft might act as a biological barrier that prevents the early contact of the oral epithelium with the root surface, hence retarding epithelial apical migration and

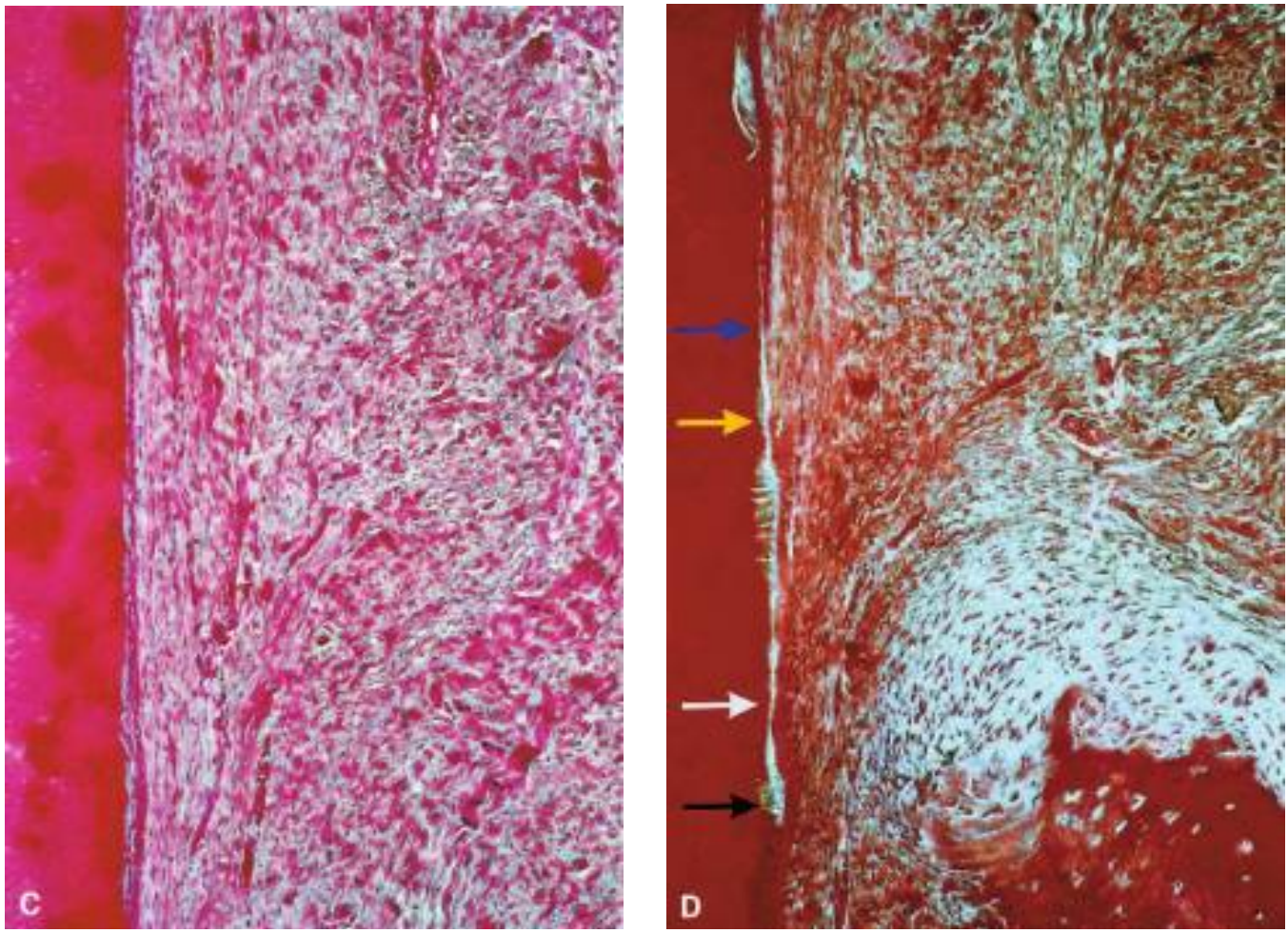

Figure 4. (continued)
C. *Higher power (white frame in A) showing epithelium extending more apically in a 1- to 2-cell layer (original magnification ×40).* ***D.*** *High power of the base of the recession site (blue frame in A) at the apical termination of root instrumentation (black arrow), showing minimal coronal bone growth (between black and white arrows), slight mineralized tissue apposition along the root surface (between black and yellow arrows), apical termination of the junctional epithelium (blue arrow), and the area of non-attached connective tissue (between blue and yellow arrows). Note the parallel arrangement of the connective tissue fibers to the root surface (adhesion) (original magnification ×40) (hematoxylin-eosin stain).*

increasing the period during which periodontal ligament cells can proliferate coronally. The biological events involved in healing in the inlay technique might be similar to those active in surgical techniques based on epithelial downgrowth retardation to obtain periodontal regeneration.[27-29]

There is conflicting evidence relative to the role of root surface preparation as a potential factor in new cementum formation.[15,30,31] Pasquinelli[15] showed that no new cementum was found in areas where the old cementum had been completely removed through root planing with hand instruments and finishing burs. These observations are supported by the work of Fukazawa and Nishimura[30] who suggested that the presence of the healthy, deep cementum layer on the root surface following light mechanical curettage might be necessary for the differentiation of repopulating cells into cementoblasts. The same authors indicated that the planed surface of dentin apparently lacks such inductive activity. These speculations are supported by human case reports showing healing through regeneration when root surface instrumentation was light and/or limited to the exposed recession area.[17,23] Controversial findings were shown by Bowers et al.[31] who demonstrated that new cementum forms equally well on old cementum, dentin, or

Table 2.

Histomorphometric Measurements of Various Components of Tissue Attachment at Recession Sites

	Combined AC Length of S and JE (mm)	AC Length of Newly Formed Bone (mm)	AC Length of Newly Formed MT Along RS (mm)	RS Exhibiting Non-Attached CT (mm)	AC Length From GM to Apical Limit of RS Instrumentation (mm)
Right maxillary first premolar	3.80	0.12	0.38	0.08	4.38
Left maxillary first premolar	3.40	0.21	0.42	—	4.03

AC = apicocoronal; S = sulcular; JE = junctional epithelium; MT = mineralized tissue; RS = root surface; CT = connective tissue; GM = gingival margin.

both old cementum and dentin in the same defect. These unsettled controversies make it difficult to attribute the lack of regeneration in the present report to the extensive subgingival root surface planing up to the marginal level of crestal bone.

The present report showed loosely organized connective tissue arranged parallel to the root surface at the base of the recession defects. This is in line with most studies evaluating mucogingival procedures alone[15-17,19,21] or in combination with regenerative materials such as enamel matrix derivative¶[32] or barrier membranes,[33,34] where fiber orientation was reported to be parallel to the tooth rather than perpendicular throughout the greatest area of the exposed root surface. This connective tissue adhesion corroborates the reparative nature of healing following periodontal plastic surgical procedures.

In the present study, the periosteum was part of the connective tissue grafts, as the donor tissues were harvested from the deep palatal area. Weng et al.[16] demonstrated regeneration with new cementum, new bone, and connective tissue attachment in surgically created dehiscences treated with deep palatal subepithelial tissue grafts completely covered by the recipient site flap in a dog model. The authors outlined the possible role of the periosteum in the regeneration process. The discrepancy between the findings of Weng et al.[16] and the results of this report that demonstrated the lack of regeneration in both specimens might be attributed to the nature of the surgically created defects and differences in species.

The postoperative changes in the relative proportions of epithelium and connective tissue contributing to an established dentogingival interface have been evaluated at various healing intervals in a rat model.[35] Listgarten et al.[35] demonstrated that the entire epithelial attachment was displaced coronally with concomitant reduction in sulcus depth and replacement of the apical portion of the junctional epithelium by a connective tissue junction of increasing dimension. This hypothesis is compatible with the findings of Caton and Zander[36] that demonstrated the presence of perforations in the junctional epithelium in 8 out of 22 healed periodontal pockets treated with periodic root planing and soft tissue curettage in a monkey model. Similarly, healing through a short junctional epithelium that stopped at the previously exposed root surface was described in a human histologic specimen 6 months following treatment of a recession defect with a connective tissue combined with partial-thickness double pedicle graft.[24] More apically, the healing was predominantly mediated through connective tissue with isolated areas of epithelium. The above-mentioned reports[24,35,36] suggest that portions of the root surface previously covered by junctional epithelium might become reattached to the surrounding connective tissue. The question whether the apical portions of the long junctional epithelium established in the present human case report can be replaced by connective tissue attachment at longer evaluation periods can only be hypothetical. Further studies are needed to confirm these histological observations and provide support for this hypothetical wound healing scheme.

In this case report, the 2 premolars were not notched at the apical extent of the recession and the coronal level of the bone crest since it was not known at the time of surgery that the teeth were to be removed. Consequently, no reference notches were

¶ Emdogain, Biora, Malmö, Sweden.

available as histological landmarks for adequate histomorphometric interpretation. However, the portion of the root instrumented during the surgical procedure was clearly recognizable for the absence of cementum. The location of the original bony crest could also be extrapolated from the histologic sections, as the exposed root surfaces were planed with curets up to the marginal level of the crestal bone during surgical root preparation.

Despite the satisfactory clinical results achieved with connective tissue grafts combined with a coronally positioned flap in the treatment of both recession defects in this case report, a question arises as to the potential of this procedure to yield some regeneration of a new attachment apparatus. The question whether a connective tissue attachment is more favorable than an epithelial attachment for the healing of recession defects or the long-term stability of the position of the soft tissue margin remains to be evaluated. Although the ability of the long junctional epithelium to act as a barrier against infection does not seem to be inferior to that offered by a normal dentogingival unit,[37,38] the objective to reconstruct *ad integrum* the lost attachment apparatus does not seem to be fulfilled with this type of periodontal plastic surgical procedure.

Within the limited information that can be drawn from this case report, it may be concluded that the use of connective tissue grafts combined with a coronally positioned flap achieves adequate clinical coverage of recession-type defects. However, under the existing clinical conditions and surgical modality applied in the 2 cases presented in this report, healing following the connective tissue grafting procedure was mediated through a long junctional epithelium along the major portion of the root with an extremely limited area of new attachment and bone formation at the base of the recessions. This type of healing could be attributed to the morphology and size of the recession defects and flap positioning at the end of surgery.

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