Histology of a Human Biopsy Section
Following the Placement of a Subepithelial Connective Tissue Graft

John F. Bruno, DDS, MS*
Gerald M. Bowers, DDS, MS**

This case report histologically examines the type of attachment that can occur after root coverage of a long-standing facial recession defect on a maxillary premolar with a subepithelial connective tissue graft. Histologic findings suggest that various types of tissue attachment, including periodontal regeneration, may occur over a recession defect after placement of a subepithelial connective tissue graft. In the present case, it was noteworthy that after 1 year the greatest area of exposed root surface was covered by a connective tissue attachment (adhesion), which had remained intact during orthodontic movement and distal drifting of the tooth. (Int J Periodontics Restorative Dent 2000;20:225–231.)

*Associate Professor, Department of Periodontology, Tufts University School of Dental Medicine, Boston, Massachusetts, and Private Practice, Springfield, Virginia.
**Professor Emeritus, Baltimore College of Dental Surgery, University of Maryland, Baltimore, Maryland.

Reprint requests: Dr John Bruno, 6120 Brandon Avenue, Springfield, Virginia 22150.

For many years, attempts have been made to cover areas of deep, wide marginal tissue recession. Predictable and reproducible esthetic results were not obtained until 1985, when Langer and Langer1 described a subepithelial connective tissue graft technique for root coverage. Others subsequently presented modifications of the original Langer and Langer technique.2-5

Although clinical predictability for root coverage has improved over the years, there is little information available regarding the nature of the soft tissue attachment to the exposed root surface. Early studies suggest that the use of pedicle flaps to cover recession defects results in a long junctional epithelial attachment.6-9 More recently, Cortellini et al10 demonstrated the potential of achieving a cementum and connective tissue attachment in a recession defect following placement of a membrane for guided tissue regeneration. In 1995, Pasquinelli11 reported that the placement of a free gingival graft could result in regeneration of bone, cementum, and connective tissue attachment on a
root surface with long-standing recession. To date, there are no reports of the histologic outcome following placement of a subepithelial connective tissue graft over recession defects. The purpose of this case report is to examine the type of attachment that can occur after root coverage of a long-standing facial recession defect with a subepithelial connective tissue graft.

Case report

A 56-year-old man in excellent health presented with generalized, severe marginal tissue recession. He was scheduled to begin orthodontic treatment that required the extraction of all 4 first premolars. He consented to the placement of a subepithelial connective tissue graft and biopsy of the maxillary left first premolar prior to the extraction. Figure 1 shows the recession defects that were scheduled for subepithelial connective tissue grafts. There was 9 mm of recession on the facial aspect of the maxillary left canine, 8 mm of recession on the facial aspect of the maxillary left first premolar, and 6 mm of recession on the facial aspect of the maxillary left second premolar. Clinical probing depths measured 2 mm on the facial aspects of the 3 teeth. Subepithelial connective tissue graft procedures were performed on the canine, first premolar, and second premolar as described by Bruno in 1994.5 Orthodontic treatment was initiated 3 months after the surgical procedures. Figure 2 shows the surgical site 6 weeks postoperative, before initiation of orthodontic treatment.

At 12 months there was marked esthetic improvement of the 3 grafted sites. There was 1 mm of remaining recession facial of the maxillary left canine, 2 mm on the first premolar, and 1 mm on the second premolar (Fig 3). The gingival tissues appeared healthy and tightly adapted to the teeth. Probing depths ranged from 1 to 2 mm, and there was no bleeding on probing (Fig 4).

Biopsy procedure

At 12 months, all 4 first premolars were extracted. The biopsy was obtained from the left first premolar as described by Bowers et al.12 Vertical incisions were made at the mesial and distal line angles of the first premolar and extended approximately 10 mm apically from the gingival margin. A horizontal incision was made, connecting the 2 vertical incisions. Vertical releasing incisions were made distal of the canine and mesial of the second premolar. Full-thickness flaps were elevated to permit access to the bone. The crown of the tooth was severed, and a groove was cut through the bone and the tooth, outlining the biopsy site. A vertical cut was made mesially and distally through the central portion of the severed crown to separate the facial biopsy from the palatal section of the root. The biopsy was carefully detached (Fig 5) and placed in 10% formalin, and the remaining root fragment was extracted. The extraction site was grafted with demineralized freeze-dried bone allograft (DFDBA) to prevent facial deformity of the alveolar ridge.

The biopsy was processed, serially sectioned, and stained with Masson's trichrome by the Oral Pathology Department, University of Maryland. Serial sections were evaluated for the type of attachment that formed over the exposed root surface.
Fig 1  Surgical area preoperatively: maxillary left canine, first premolar, and second premolar.

Fig 2  Surgical area 6 weeks postoperative, prior to orthodontic therapy.

Fig 3  Surgical area immediately prior to the biopsy procedure, 1 year after the graft procedure and 9 months after the initiation of orthodontic therapy.

Fig 4  Probe demonstrates less than 3 mm of probing depth immediately prior to the biopsy procedure.

Fig 5  Biopsy of left first premolar, after sectioning of the facial fragment and prior to removal.
Representative sections of the histologic results are shown in Figs 6 to 10. There appeared to be some coronal apposition of new bone at the base of the defect (Fig 6). Figure 7 clearly demonstrates the formation of new bone, cementum, and periodontal ligament, which ranged from 0.5 to 1 mm from the root-planing groove. The newly formed cementum was thin, cellular, and had formed over a thin layer of old cementum. Sharpey's fibers were observed inserting into the newly formed bone and cementum. The periodontal ligament was loosely organized and arranged parallel to the root.

The major portion of the pathologically exposed root surface was covered by connective tissue, which ranged from 3 to 5 mm in length (Fig 8). The connective tissue appeared to be in intimate contact with the irregular dentinal surfaces (Fig 9). The connective tissue was highly vascular and contained adipose tissue. The nature of the attachment was suggestive of a connective tissue adhesion.

Figure 10 delineates the extent of the migration of the junctional epithelium. In some sections, the epithelium was located totally on old cementum. In other sections, the epithelium, one to two cells in thickness, had migrated onto the prepared dentinal surfaces (Fig 11). There was no evidence of replacement resorption or ankylosis in any of the serial sections.
Fig 9  Note the intimate contact of the parallel fibers of the connective tissue (CT) and the irregular dentin surface (D). (Original magnification × 51; Masson's trichrome stain.)

Fig 10  Apical location of the junctional epithelium (JE) in this section is on old cementum (OC). (Original magnification × 8; Masson's trichrome stain.)

Fig 11  Higher magnification of Fig 10 shows the apical extent of the junctional epithelium (JE) on old cementum (OC). (Original magnification × 51; Masson's trichrome stain.)

Discussion

Placement of a subepithelial connective tissue graft resulted in marked esthetic improvement of 3 adjacent recession defects. Complete root coverage, however, was not achieved in any of the sites. Residual recession of 1 to 2 mm could have been related to the severity of the defects and the reduced preoperative height of the interproximal tissues (Fig 1). Close root proximity of the adjacent teeth and distal drifting of the first premolar also compromised coronal flap repositioning, which could have resulted in failure to obtain complete root coverage. It was interesting to note that the postoperative levels of the grafted sites were not affected by the orthodontic movement. The same amount of root exposure noted at 6 weeks was present 1 year later (Figs 2 and 3).

Histologic findings suggest that subepithelial connective tissue grafts may heal by various modes of attachment to the tooth. At the base of the recession defect, attachment occurred by periodontal regeneration (new bone, cementum, and periodontal ligament). This was encouraging since surgical procedures on the facial surface frequently result in postsurgical loss of the remaining thin cortical plate. In this case, there was a slight gain in the bone height, as well as the formation of new cementum and periodontal ligament. This finding supports the case reports of Cortellini et al and Pasquinelli, who observed the
formation of new bone, cementum, and periodontal ligament over recession defects following the use of guided tissue regeneration and free gingival grafts, respectively. It is meaningless to compare the amount of regeneration of various tissues in case report documentation since the number of variables is limitless. Nevertheless, it is interesting that in all 3 case reports, varying amounts of periodontal regeneration were observed. Therefore, it could be speculated that the potential for some regeneration of a new attachment apparatus exists following periodontal plastic surgery procedures. Comparative studies are necessary to determine if there is a histologic advantage of one technique over another.

It was interesting—and surprising—that after 1 year, the greatest surface area of exposed root surface was covered by a connective tissue attachment (adhesion). Bowers and coworkers reported small amounts of connective tissue adhesion following the placement of DFDBA in human intrabony defects. In their study, biopsies were obtained at 6 months, and the authors speculated that the sites of connective tissue adhesion were possibly an interim stage of new cementum formation. In the present report, connective tissue attachment had persisted for 1 year without converting to cementum and without adversely affecting the root surface. The long-term fate of connective tissue attachment still remains unclear. The fact that the connective tissue attachment remained intact during orthodontic movement and distal drifting suggests that such an attachment or adhesion is resistant to recurring recession.

There is controversy regarding whether original root cementum is necessary for the formation of new cementum. Fukazawa and Nishimura speculated that original root cementum may be necessary to induce repopulated cells into cementoblasts. They also suggest that dentin lacks this capability. Pasquinelli observed that no new cementum formed where old cementum had been removed. Conversely, Bowers and Middleton reported, in a human histologic study of 320 grafted (DFDBA) and nongrafted sections, that cellular cementum was just as likely to form over dentin as over old cementum. They noted that on occasion, new cementum would completely cover the severed dentinal surfaces of vitally submerged roots. In this case report, new cementum formed over original root cementum and paralleled the amount of new bone formation. It is possible that with increased bone formation, new cementum would also have formed over the dentinal surface.

Subepithelial connective tissue was used in this case report to cover multiple deep, wide facial recession defects on 3 adjacent teeth. Root coverage was incomplete, but there was a marked increase in gingival height and improved esthetics. Findings suggest that the amount of soft tissue coverage achieved following subepithelial connective tissue grafting can be maintained during orthodontic movement. Histologic findings indicate that various types of tissue attachment, including periodontal regeneration, may occur over a recession defect after placement of subepithelial connective tissue grafts. Further histologic studies/case reports are necessary before definitive conclusions can be drawn regarding the nature of the attachment that can be anticipated following periodontal plastic surgery procedures.

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