Evaluation of Human Recession Defects Treated with Coronally Advanced Flaps and Either Enamel Matrix Derivative or Connective Tissue. Part 2: Histological Evaluation

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Background: A number of surgical procedures are effective in covering denuded root surfaces. The first paper in this series evaluated the subepithelial connective tissue graft and the coronally advanced flap with enamel matrix derivative (EMD). That paper revealed no significant difference in the percent of root coverage between the two treatments (P= 0.82). There is limited human histological evidence of the type of attachment achieved with these types of procedures. This paper presents a human case report detailing the histological nature of the attachment of these two treatments to the root surfaces previously exposed by recession.

Methods: One patient presented with two hopeless teeth that were randomized to receive either a subepithelial connective tissue graft or a coronally advanced flap plus EMD. The surgery was accomplished in accordance to the protocol previously described. The teeth and a small collar of tissue were removed at 6 months and underwent histological analysis.

Results: Histological evaluation of the subepithelial connective tissue graft revealed a connective tissue attachment between the tooth and graft, and no histological evidence of cementum, bone, or periodontal ligament (PDL) and, therefore, regeneration. In addition, there appeared to be some resorption of the dentin adjacent to the graft. Histological evaluation of the coronally advanced flap with EMD revealed new cementum, organizing PDL fibers and islands of condensing bone at a constant distance from the root surface.

Conclusions: The subepithelial connective tissue graft in this study was found to have adhered to the root surface primarily by a connective tissue attachment with some evidence of root resorption. The coronally advanced flap with EMD was found histologically to have all the tissues necessary for regeneration: new cementum, organizing PDL fibers, and islands of condensing bone. These histologic sections strongly suggest that enamel matrix derivative works in a biomimetic fashion by mimicking the natural process of tooth development. *J Periodontol 2003;74:1126-1135.*

KEY WORDS

Biometry; comparison studies; enamel matrix derivative; follow-up studies; gingival recession/surgery; gingival recession/therapy; grafts, connective tissue; periodontal regeneration; proteins, enamel matrix/therapeutic use; surgical flaps.

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Recession defects around teeth are usually treated to achieve patient-centered outcomes such as a reduction in root surface sensitivity, ease in plaque control, treatment or prevention of root caries, and esthetic concerns regarding excessive tooth length and abnormal gingival contours. A variety of surgical procedures have been found to be effective in covering denuded root surfaces, but current literature indicates that the subepithelial connective tissue graft offers increased predictability.[1-6] The coronally advanced flap has also been shown to be effective in covering recession type defects.[7-11] Although not reported to be as predictable as the subepithelial connective tissue graft, the coronally advanced flap is attractive to both the patient and the clinician because it does not require a second surgical site to harvest donor tissue. The first paper in this series[12] evaluated the use of subepithelial connective tissue plus coronally positioned flap and enamel matrix derivative (EMD) plus coronally positioned flap in treatment of human recession defects. There were no statistically significant differences in clinical attachment gain, root sensitivity, probing depth, or any of the other evaluated parameters with the exception of healing at 1 week, self-reported discomfort, and width of keratinized gingiva. There was no significant difference in the percent of root coverage between the two groups ($P = 0.82$). At the end of 12 months, 93.8% of the root surfaces treated with subepithelial connective tissue grafts were covered and 95.1% of the root surfaces treated with coronally advanced flap plus EMD were covered. Both treatment groups demonstrated an average gain of attachment of 4.5 mm (range 4 to 8 mm). One hundred percent root coverage was obtained 89.5% of the time with the coronally advanced flap with EMD and 79% of the time with the subepithelial connective tissue graft. Within the limitations of that paper, the results indicated that the addition of EMD to the coronally advanced flap resulted in similar root coverage as compared to the subepithelial connective tissue graft without the morbidity and potential clinical difficulties associated with the donor site surgery. Trombelli[13] stated that the goal of root coverage grafts was to recreate the functional and esthetic morphology of the mucogingival complex and regenerate the lost attachment apparatus including the formation of new cementum with inserting connective tissue fibers and regeneration of alveolar bone. The first paper in this series[12] demonstrated that both the coronally advanced flap with EMD and the subepithelial connective tissue graft successfully recreated both a functional and esthetic mucogingival complex over denuded root surfaces. This paper will explore the second requirement, the type of attachment achieved with the subepithelial connective tissue graft versus that achieved with the coronally advanced flap with EMD. One human case report detailing the histological nature of the attachments to root surfaces previously exposed by recession achieved by these two treatments forms the basis for this examination. Therefore, the aim of this present investigation is to assess the quality and nature of new tissue attachment to a previously denuded root surface (recession defect) following treatment with either coronally advanced flap with EMD or a coronally advanced flap with subepithelial connective tissue.

MATERIALS AND METHODS

Of the 20 patients enrolled in a clinical study,[12] one patient presented with two hopeless teeth, classified as Miller Class IV,[14] which were scheduled for extraction. This patient was 29 years old at the time of the study, a non-smoker in good health, and had no contraindications for periodontal surgery. The maxillary right lateral incisor (#7) was periodontally and endodontically hopeless and the left central incisor (#9) was not periodontally strong enough to act as an abutment for either a fixed or removable bridge. Before any therapy was accomplished, the protocol was approved by an institutional review board and an informed consent form was discussed with the patient and signed by the patient. The patient had two other teeth (#6 and #11) that qualified for inclusion into the study. At the surgical visit, the envelope containing the computerized randomization schedule was opened and teeth #6 and #7 were randomized to receive a coronally advanced flap plus EMD[‡] and teeth #9 and #11 were assigned a subepithelial connective tissue graft.

Surgical Procedure

All four of the teeth were treated in accordance with the surgical procedure outlined in the previous paper[12] with the exception that a notch was placed into the root surface at the preoperative free gingival margin on the right lateral incisor (#7) and on the left central incisor (#9), and a notch was placed into the root surface at the alveolar bone crest on the left central incisor. The right lateral incisor had no facial cortical plate and the end of the root had been removed through an apicoectomy. Because there was no cortical plate on tooth #7, the notch was placed at the most apical portion of the root. The left central incisor treated with subepithelial connective tissue healed without complications (Figs. 1A through 1D). The right lateral incisor treated with coronally advanced flap plus EMD was found to have a fenestration in the flap when the patient was seen at the 1-week postoperative appointment (Figs. 2A through 2E). The patient was instructed to clean this area with a cotton swab with 0.12% chlorhexidene gluconate[§] for the first 4 weeks. The patient followed the postoperative treatment outlined in the previous paper.[12] At 6 months, the two hopeless teeth were extracted with a small collar of tissue (Figs. 1E and 2E). Following local anesthesia, a full thickness incision was made outlining the collar of tissue to be removed. A full thickness flap was then reflected apically, mesially and distally. A high-speed bur was used to extend the incisions through the bone and into the root. The tooth with the block section was gently elevated and removed without disturbing the collar of tissue. The teeth and block sections were immediately placed into separate containers filled with 10% neutral buffered formalin. The extraction sites were grafted with demineralized freeze-dried bone allografts and a collagen barrier membrane. To further minimize the post-surgical defect and preserve the ridge, a connective tissue ridge augmentation was performed 2 months following the extraction.

After fixation in 10% neutral buffered formalin, the samples were placed in a decalcifying solution until decalcification was complete as determined by x-ray.

‡ Emdogain, Biora AB, Malmo, Sweden.
§ Peridex, Procter & Gamble, Cincinnati, OH.

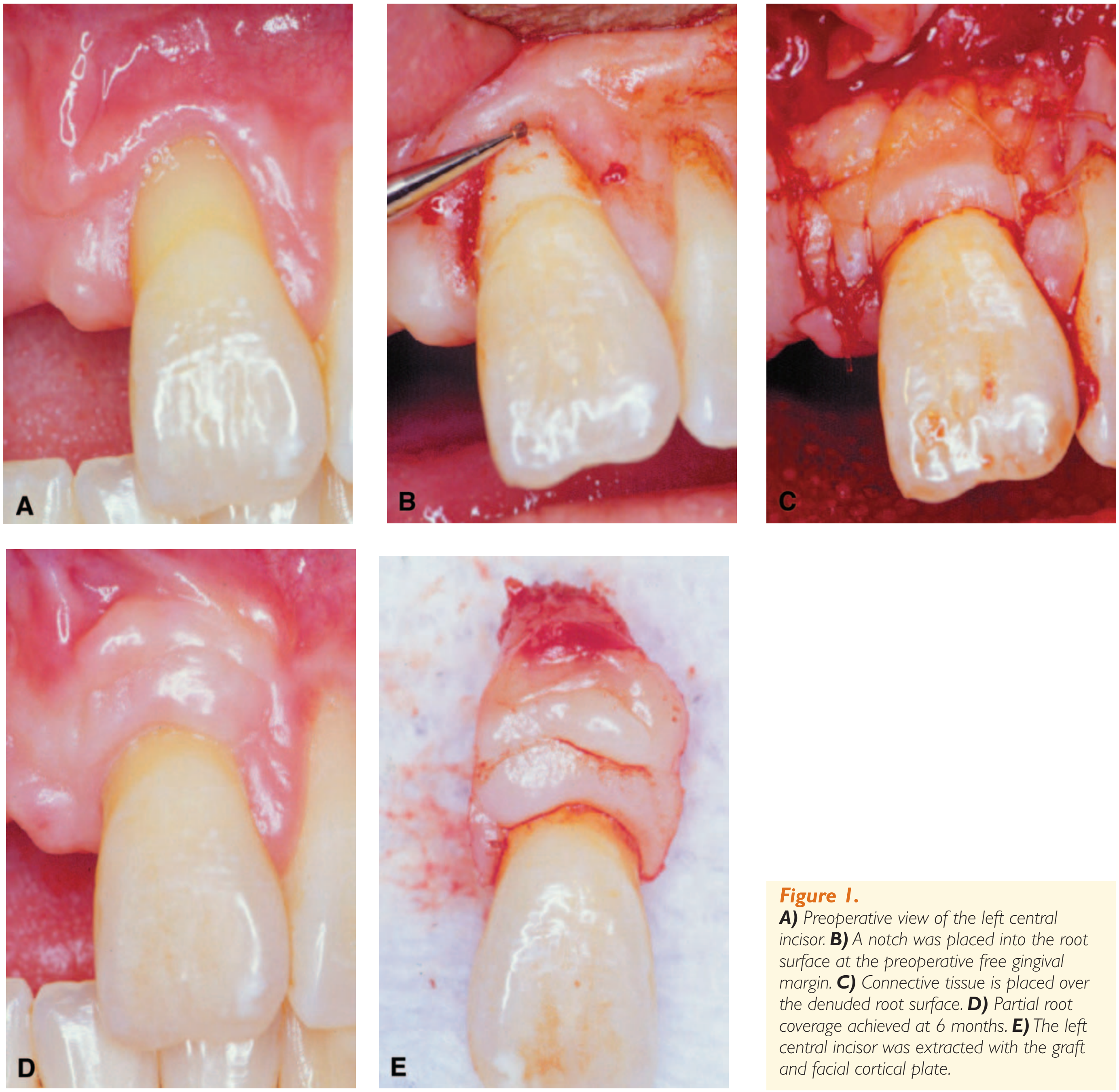

Figure 1.
A) *Preoperative view of the left central incisor.* ***B)*** *A notch was placed into the root surface at the preoperative free gingival margin.* ***C)*** *Connective tissue is placed over the denuded root surface.* ***D)*** *Partial root coverage achieved at 6 months.* ***E)*** *The left central incisor was extracted with the graft and facial cortical plate.*

Specimens were then processed through different grades of alcohol and xylene and embedded in paraffin. Each specimen was embedded parallel to the long axis of the tooth. Sectioning was done on a microtome. Every second section was cut at 3 microns, then stained with routine Harris hematoxylin and eosin stain and histologically evaluated. The examiner was masked to the treatments received.

RESULTS

Histological evaluation of the left central incisor treated with the coronally advanced flap and subepithelial connective tissue demonstrated a long junctional epithelium ending at the coronal extent of the notch representing the preoperative gingival margin. Below the apical extent of the junctional epithelium, connective tissue was found adjacent to the dentin in the notch. Bundles of non-inflamed connective tissue extended laterally to the oral epithelium and composed the bulk of the tissue. Inside the coronal notch, the dentin exhibited a roughened appearance indicative of a resorption process. In the notch made at the alveolar crest, cementum was found in the apical two-thirds of the notch and connective

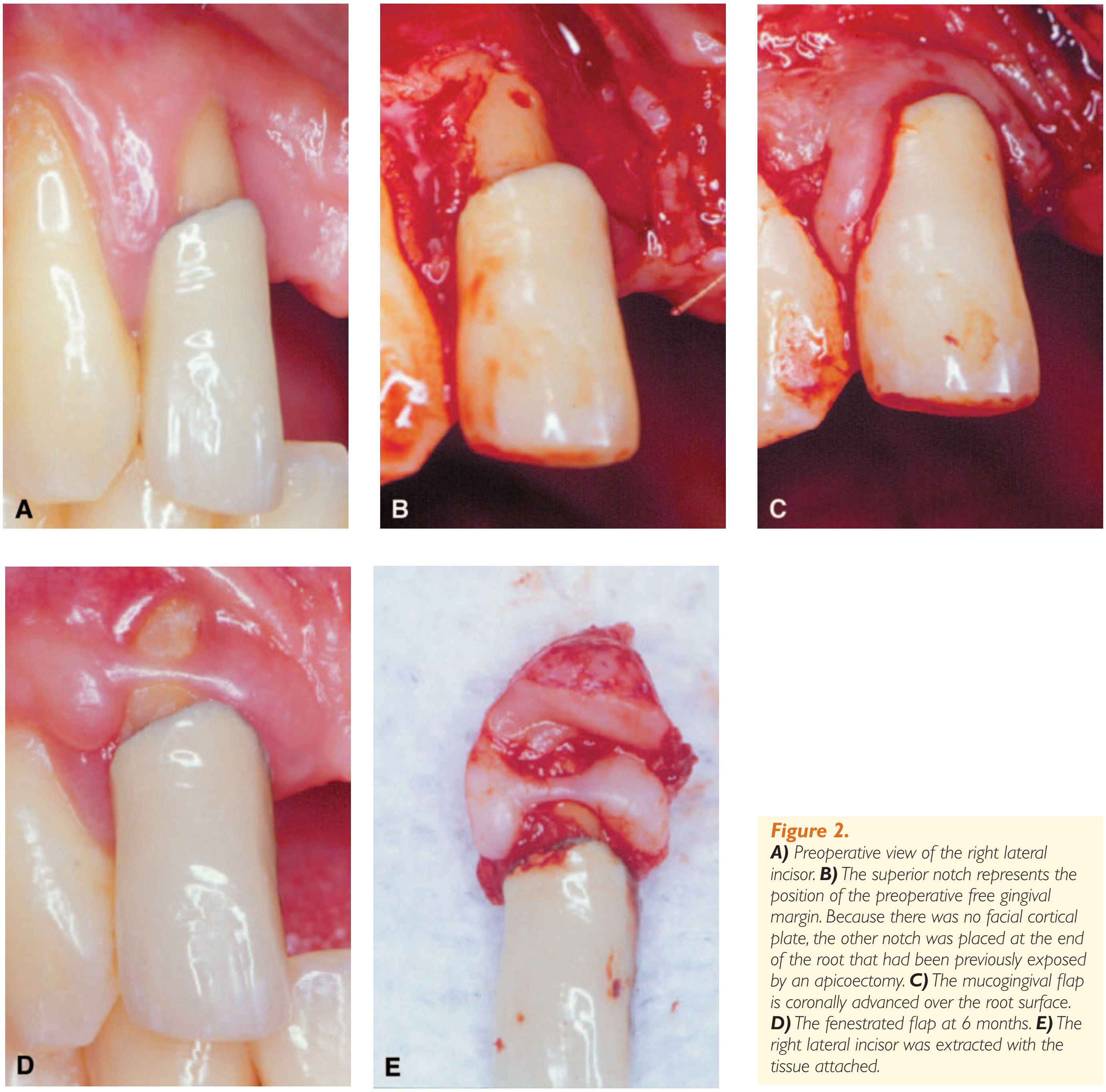

Figure 2.
A) *Preoperative view of the right lateral incisor.* ***B)*** *The superior notch represents the position of the preoperative free gingival margin. Because there was no facial cortical plate, the other notch was placed at the end of the root that had been previously exposed by an apicoectomy.* ***C)*** *The mucogingival flap is coronally advanced over the root surface.* ***D)*** *The fenestrated flap at 6 months.* ***E)*** *The right lateral incisor was extracted with the tissue attached.*

tissue was seen in between the two notches on the tooth. An overview of this histology is depicted in Figure 3.

For closer examination of the quality of the attachment, a series of histological sections were made beginning coronal to the notch at the original free gingival margin and moving apically to just below the notch at the alveolar crest. The section identified as Figure 4 clearly illustrates the junctional epithelium ending just above the notch placed at the gingival margin. From that point moving apically to the notch placed at the alveolar crest, Figure 5 illustrates evidence of a small amount of new cementum formation in the notch, but no new bone formation.

Figure 6 shows higher power magnifications of the nature of the tissue attachment at the lower notch. In these sections there is evidence of new cementum formation along with connective tissue but no evidence of new bone formation. Overall, the analysis of the histology reveals connective tissue present between the tooth and the oral epithelium, and no histological evidence of periodontal regeneration.

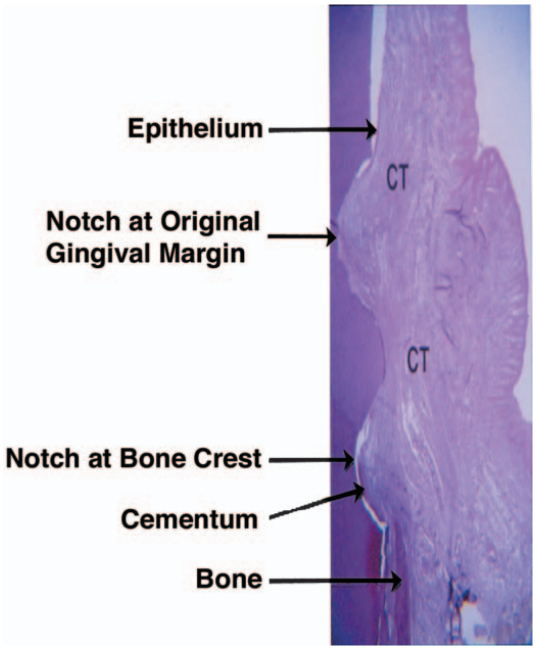

Figure 3.
A low power (25×) view of the subepithelial connective tissue graft.

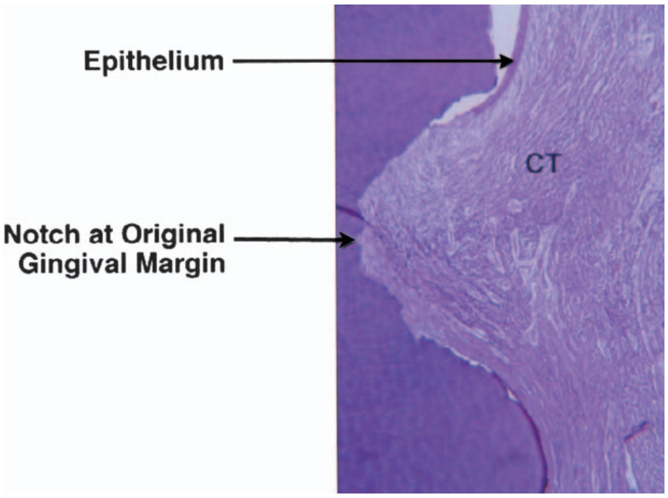

Figure 4.
A low power (250×) view of the notch at the original gingival margin with the junctional epithelium ending to the coronal aspect of the notch.

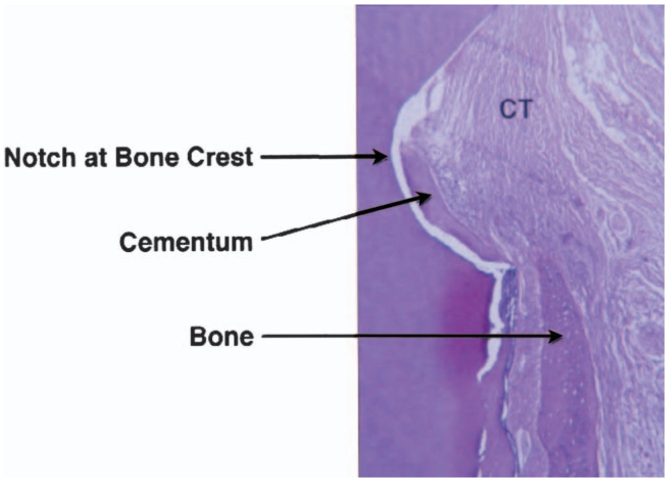

Figure 5.
A low power (250×) view of the notch at the original alveolar crest. Cementum formation is seen in the notch.

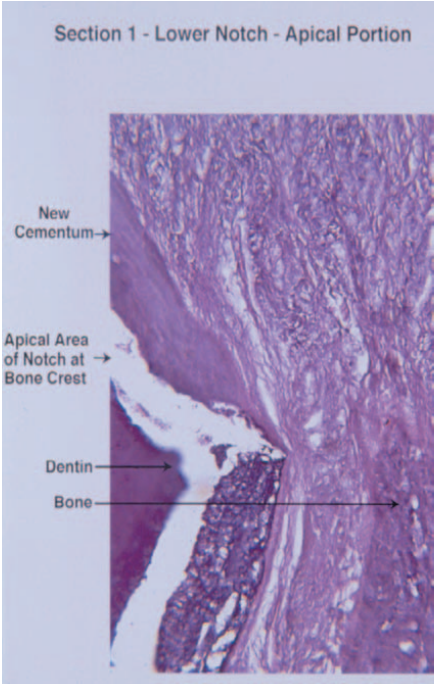

Figure 6.
A higher power (625×) view of the apical portion of the lower notch. New cementum and connective tissue is seen, but there is no evidence of new bone formation.

Histological evaluation of the right lateral incisor treated with the coronally advanced flap and EMD was complicated by the presence of a fenestration of the mucogingival flap (Fig. 2D) in the region of the notches rendering them useless as histological markers. Despite these challenges, an overview of the histology is presented in Figure 7.

Figure 8 shows a higher magnification of the quality of the attachment, clearly illustrating the presence of new cementum, organizing PDL fibers, and islands of condensing bone. In this section, the PDL fibers are running parallel to the root surface.

DISCUSSION

The purpose of this study was to histologically evaluate the type of attachment that was achieved over previously exposed root surfaces using the subepi-

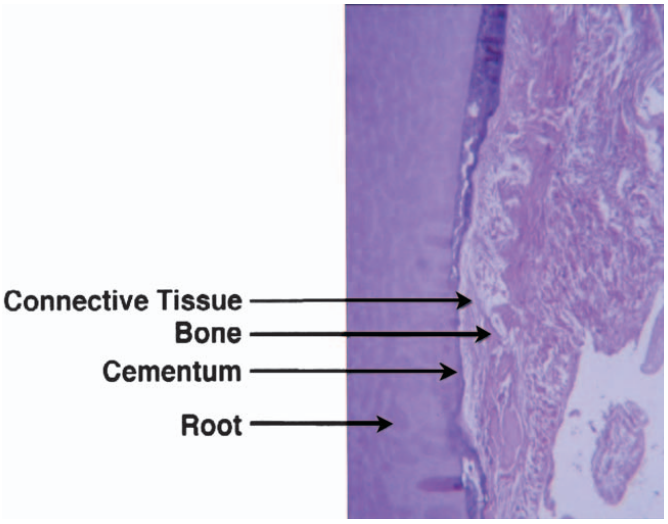

Figure 7.
A low power (250×) view of the coronally advanced flap with EMD. Note the islands of condensing bone that have formed at a uniform distance from the root surface. New cementum and an organizing PDL are also evident.

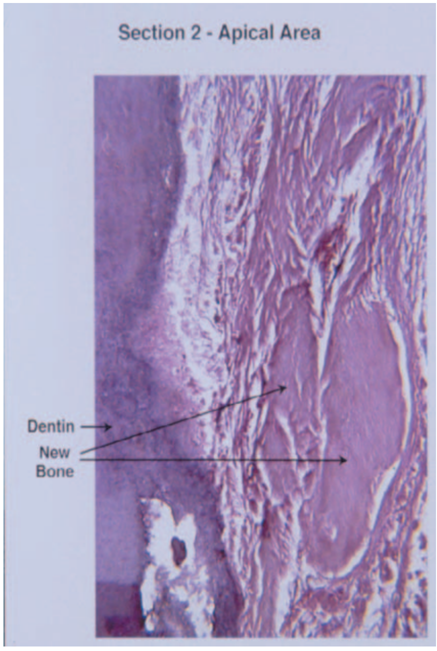

Figure 8.
A high power (625×) view of the apical area of the section demonstrating the formation of new bone along the root surface.

thelial connective tissue graft and the coronally advanced flap with EMD. For obvious reasons, there is little information available on the type of attachment achieved in humans relating to root coverage grafts. The amount of root coverage achieved on both of the study teeth was limited due to the fact that we were treating hopeless teeth with Miller's Class IV[14] recession defects. One would expect only limited root coverage in these types of defects in the best of cases. Partial root coverage was achieved on both teeth, but the flap on the lateral incisor experienced a fenestration that complicated the histological evaluation and the clinical outcome. The fenestration may have occurred because of a variety of clinical factors: there was no bone on the facial or mesial aspect of the tooth from the cemento-enamel junction to beyond the root apex; the tissue was very thin; there was Class II mobility of the tooth;[15] and, the topography of the defect required the flap to be extensively repositioned coronally. Even considering these limitations, important information regarding the type of attachment of root coverage grafts was provided. The histological analysis of the subepithelial connective tissue graft found the connective tissue attachment intimately opposed to the dentin with a long junctional epithelium limited to the most coronal portion of the graft. There were large non-inflamed connective tissue bundles making up the bulk of the new tissue and the new tissue extended above the original gingival margin. Thus, there was histological evidence for greater root coverage that reinforced the clinical findings. However, this new coronal tissue resulted in a long junctional epithelium to the root surface and evidence of root resorption in the notched area. There was some cementum formation (likely reparative cementum) seen in the notch made adjacent to the alveolar crest, most likely due to the acute trauma of making the notch, but there was no evidence of a new attachment apparatus comprised of new cementum, bone, nor inserting PDL fibers. In fact, the alveolar crest level did not appear to be altered in spite of the open flap procedure.

A review of the literature pertaining to human histology of subepithelial connective grafts reveals substantial variability from case to case. Goldstein et al.[16] reported regeneration of the attachment apparatus including new bone, cementum, and PDL following a root coverage procedure using a subepithelial connective tissue graft that included periosteum. Bruno and Bowers[17] reported on the histological results of a human biopsy 1 year following coverage of a denuded root surface with a subepithelial connective tissue graft. They found that only the apical portion of the denuded root surface healed by regeneration (new bone, cementum, and PDL) and that the majority of the defect healed by connective tissue adhesion. Majzoub et al.[18] reported on two maxillary bicuspids, which were extracted for orthodontic reasons 1 year after a subepithelial connective tissue graft was placed to cover Miller Class I recession defects. They concluded that the graft healed with a long junctional epithelium. Harris[19] showed no evidence of regeneration 6 months postoperatively in two recession defects treated with a connective tissue graft covered by a partial thickness double pedicle graft.

In a different report Harris[20] demonstrated new bone, cementum, and connective tissue attachment coronal to the presumed location of the preoperative gingival margin at 5 months. He suggested that the difference in the attachment might have been related to the disparity in the defect size in the two case reports, 2 and 3 mm versus 4 mm in the report where regeneration occurred. Harris postulated that defects with greater recession depth may have greater opportunity for regeneration.

The type of attachment achieved over teeth with recession defects with grafts other than subepithelial connective tissue grafts has also been demonstrated to be extremely variable. Pasquinelli[21] demonstrated histologically that the attachment of a thick free autogenous graft included new cementum with the insertion of Sharpey's fibers. Some new bone was seen providing evidence of partial regeneration, although strong conclusions were difficult to draw, because there were no preoperative reference notches. Cortellini et al.[22] reported regeneration over the denuded root of a mandibular canine following guided tissue regeneration (GTR) using an expanded polytetrafluoroethylene (ePTFE)‖ membrane. New cementum, Sharpey's fiber attachment, and new bone were seen histologically. The orientation of the fibers was parallel to the tooth rather than perpendicular. In a more recent report, Harris[23] reported on a histological evaluation of four teeth with recession defects treated with GTR using a polylactic acid membrane.¶ No regeneration was found and three of the defects treated healed with a long junctional attachment. Vincenzi et al.[24] presented histology consistent with regeneration when covering a denuded root by using a resorbable membrane made of a copolymer of glycolide and lactide.‖ Parma-Benfenati and Tinti's paper[25] claimed regeneration using a titanium-reinforced ePTFE membrane.‖ Neither of the last two studies placed a reference notch at the gingival margin. Richardson and Maynard[26] published a case report on a biopsy of acellular dermal matrix,# which was placed on a periodontally healthy tooth without gingival recession. The attachment was described as a fibrous tissue apposition to the root surface.

EMDs, produced by Hertwig's epithelial sheath, play an important developmental role in cementogenesis and in the development of the periodontal attachment apparatus.[27-30]

It is postulated that the mechanism of action following the application of EMD on a root surface is that it promotes selective cell repopulation during the early stages of periodontal healing.[31] Other studies have documented that EMD enhances proliferation, differentiation, and migration of osteoblast and PDL cells.[32-35]

Histological confirmation of new cementum, after application of EMD, was also reported by Mellonig[36] in the treatment of intrabony periodontal defects. Likewise, Sculean et al.[37] reported the formation (at 6 months) of new cementum with inserting collagen fibers in two intrabony defects, and in one of the two specimens there was bone re-formation. They concluded that EMD may enhance the formation of a new connective tissue attachment on a previously diseased root surface. Yukna and Mellonig[38] also provided histological proof of principle that EMD could result in regeneration on previously diseased root surfaces in intrabony defects.

Heijl et al.[39] reported on the histological results of a coronally advanced flap with EMD over an experimentally created recession defect on a mandibular incisor. At 4 months, new acellular cementum, PDL, and alveolar bone were found. These investigators suggested that since denuded root surfaces had been successfully covered clinically and regeneration had been demonstrated histologically in humans, EMD could be used as an adjunct to promote regeneration. Rasperini et al.[40] demonstrated histological evidence of regeneration after treatment of gingival recession with a subepithelial connective tissue graft plus EMD. Based on this body of knowledge it seemed reasonable to evaluate the addition of EMD to the coronally advanced flap.

The histological analysis of the coronally advanced flap plus EMD was compromised by the fact that the postoperative flap fenestration rendered the reference notches useless. Unfortunately, without reference points it is impossible to prove beyond a doubt exactly where on the root surface the slices were taken. Intraoperative photographs, however, demonstrate that there was no bone on the facial and mesial of this tooth and this is where the slices were taken. In addition, this tooth had received an apicoectomy many years prior to extraction, which had left the tooth without any bone at the apex. Based on these clinical observations of no bone present, one would have to conclude that any bone seen histologically would represent new bone formation. Although unfortunate, the absence of reference notches is not unusual; approximately 50% of the human histological evaluations of root coverage grafts reported in the literature lack reference notches.

The analysis demonstrates cementum lining the treated root surface that was primarily cellular in nature. Although EMD is purported to foster the development of acellular cementum, it is not uncommon to find both cellular and acellular forms deposited on both old cementum and dentin.[40,41] In addition, new cementum that occurs with bone grafts is usually cellular in nature.

‖ W.L. Gore & Associates, Inc., Flagstaff, AZ.
¶ Guidor USA, Bensenville, IL.
# Life Cell Corp., The Woodlands, TX.

Islands of condensing bone were observed at a uniform distance from the root surface. This is a very interesting finding and, to the best of the authors' knowledge, this is the first time that this type of *de novo* bone formation in humans has been reported in the literature. All other reports of bone formation in conjunction with any type of root coverage graft represent an extension of or apposition to the existing alveolar crest. As demonstrated photographically (Fig. 2B), there was no existing alveolar crest adjacent to the root surface under examination. The only time in nature that bone forms at a fixed distance from the root surface is during tooth development. These histologic sections strongly suggest that enamel matrix derivative works in a biomimetic fashion by mimicking the natural processes of tooth development (Fig. 7).

Connective tissue was observed running parallel between the cementum on the root surface and the islands of condensing bone. Presumably, this tissue is organizing PDL fibers. A requirement of regeneration is functionally oriented PDL fibers, but most histological studies evaluating root coverage procedures, whether it is with a coronally advanced flap, GTR, connective tissue, or EMD, report that fiber orientation was predominantly parallel to the tooth rather than perpendicular.[17,21-23,40] The Majzoub et al.[18] histological case report showed loosely organized connective tissue arranged parallel to the root surface at the base of the recession defect treated by a connective tissue graft. Finding PDL fibers parallel to the root surface is also consistent with histological findings by Bowers et al.[41,42] of new attachment after bone grafts. Mellonig[36] and Yukna and Mellonig[38] also found parallel PDL fibers when performing regenerative therapy with EMD in intrabony defects. Perhaps a longer period of healing is necessary for the fibers to properly orient themselves, particularly in the present report since the bone tissue was also at an immature stage.

Further histological studies are needed to evaluate and compare the quality and amount of regeneration after treatment with EMD and coronally advanced flap compared to other root coverage grafts. This histological case report represents only two specimens, and additional biopsies are necessary to confirm that regeneration is a frequent finding following the use of a coronally advanced flap plus EMD. An evaluation at different time points to better understand the sequence of tissue development after treatment would also be useful.

CONCLUSION

The first paper in this series[12] demonstrated that the addition of EMD to the coronally advanced flap resulted in similar root coverage as compared to the connective tissue graft without the morbidity and surgical challenges associated with the donor site surgery. In addition, the coronally advanced flap with EMD resulted in a more esthetic outcome compared to the connective tissue graft.

This paper presents the results of the histological analyses of two teeth with recession, one defect covered with a coronally advanced flap plus EMD and the other covered with a subepithelial connective tissue graft. The subepithelial connective tissue graft was found to have connective tissue intimately opposed to the root dentin apical to the junctional epithelium. There was histological evidence of root coverage above the original free gingival margin reinforcing the clinical findings previously reported.[12] No evidence of regeneration was found and evidence of root resorption was observed. Histological sections of the coronally advanced flap plus EMD demonstrated the presence of cementum, interspersed connective tissue (interpreted to be organizing PDL by its location), and islands of condensing bone found at a fixed distance from the root surface. Even though the results are open to interpretation because of the absence of reference notches on the tooth treated with coronally advanced flap plus EMD, there is no doubt that the healing mechanism following that treatment comes much closer to regeneration than did the coronally advanced flap with the subepithelial connective tissue graft. The histological sections are from the same patient with the surgery performed on the same day, by the same clinician, yet something very different is occurring histologically. The results suggest that EMD may possess potential for enhancing periodontal regeneration of coronally advanced flaps over denuded root surfaces.

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