Use of the Carbon Dioxide Laser in Retarding Epithelial Migration: A Pilot Histological Human Study Utilizing Case Reports

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PREDICTABLE REGENERATION OF TOOTH-SUPPORTING tissues lost to periodontal disease is the aim of periodontal therapy. Often the result of conventional treatment is healing with a long junctional epithelium along the root surface and little regeneration of the complete attachment apparatus. The purpose of this pilot study was to evaluate whether de-epithelialization with a CO_2 laser at the time of flap surgery and at 10-day intervals over the first 30 days of healing has the potential to enhance the formation of a connective tissue attachment. Six mandibular incisors in two patients were selected for the study. Each patient received oral hygiene instruction and initial therapy prior to surgery. The teeth were splinted together, open flap debridement was performed on all teeth, a notch was placed on the roots at the height of the crest of the alveolar bone, and the flaps were sutured in place. The test side received controlled de-epithelialization of the outer (oral) gingiva with the carbon dioxide laser, and the inner gingival flap. The de-epithelialization was repeated on the test side at 10, 20, and 30 days postsurgically. Controls received open debridement only. Block sections were taken at 90 days and processed for histologic analysis. The results showed that for both patients, junctional epithelium (JE) was formed on both test and control teeth. In all control teeth, the JE extended the entire length of the root to the base of the reference notch. On the test side (laser treated) in one patient, the notch was filled with connective tissue and limited repair cementum. This finding was not seen in any control teeth. This is the first reported observation of human histologic evaluation utilizing the CO_2 laser for de-epithelialization and may warrant further study. *J Periodontol 1995;66:197–204.*

Key Words: Surgical flaps; connective tissue attachment; laser surgery; epithelial attachment.

A primary objective of periodontal surgical therapy is the establishment of a new connective tissue attachment to a root previously exposed to periodontal disease. Unfortunately, attempts at achieving this goal often resulted in a rapid epithelial migration and a long junctional epithelium precluding new connective tissue attachment.[1,2]

In view of these findings, numerous techniques have been attempted to retard epithelial downgrowth. Chemical means such as phenol, citric acid, antiformin, and sodium hypochlorite have been tried with varying degrees of success.[3-6] Other attempts to delay epithelial migration have included cryosurgery,[7] open denudation,[8] and free soft tissue grafting as a barrier.[9]

Melcher postulated that if epithelium and gingival connective tissue were excluded from the healing surgical site then progenitor cells migrating from the periodontal ligament would have the potential to form new connective tissue attachment.[10] Nyman and co-workers, using a millipore filter as a barrier, were able to demonstrate in animal and human studies new attachment on previously diseased root surfaces.[11,12] This led to the development of expanded polytetrafluorethylene (ePTFE) membranes which have been used effectively to prevent epithelial migration, resulting in substantial new connective tissue attachment.[13]

The use of the CO_2 laser in periodontics has been limited to soft tissue surgery, resective procedures (gingivectomy and gingivoplasty), and frenectomies (for review see 14). In a preliminary study Rossmann et al. have shown that the CO_2 laser will effectively remove the epithelium without causing damage to the underlying connective tissue.[15] In a

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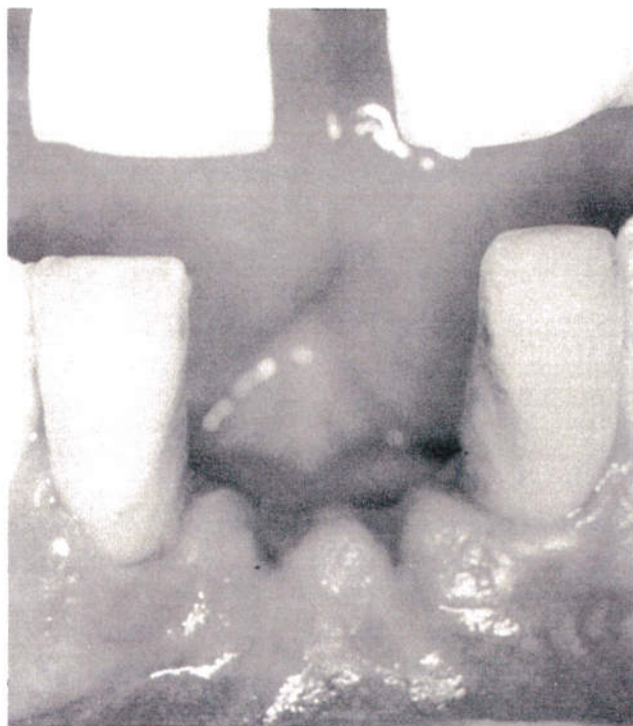
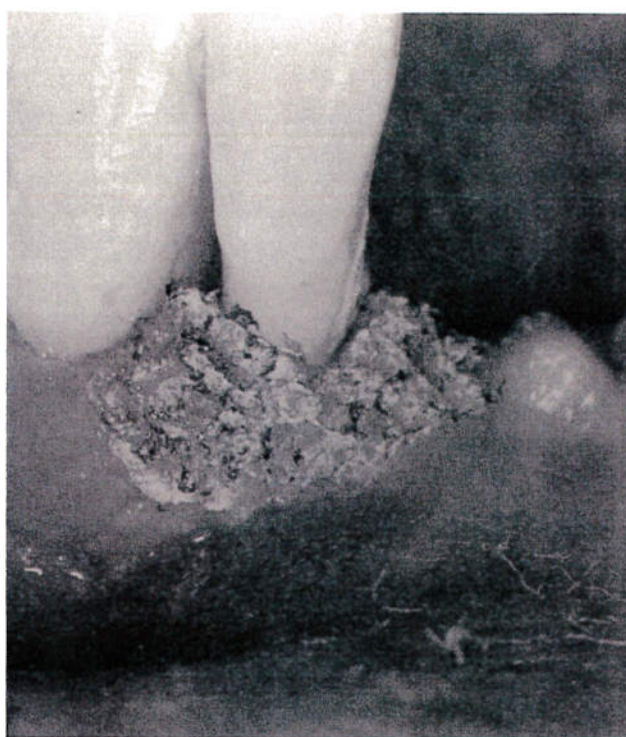Figure 1. Preoperative surgical appearance of the test (26) tooth on patient 1.

Figure 2. De-epithelialization of tooth 26 following the use of the CO_2 laser in patient 1.

follow-up animal study, Rossmann et al. showed that de-epithelialization with the laser retards epithelial downgrowth following periodontal surgery for up to 14 days longer than conventional flap techniques.[16]

The delayed epithelialization found in laser wound healing has been investigated in the dental and medical literature. Hall found that scalpel wounds in rat skin were epithelialized within 48 hours, but CO_2 laser wounds required 7 days to completely epithelialize.[17] Fisher et al., studying the healing of canine buccal mucosa, confirmed that delayed epithelialization in conjunction with a reduced inflammatory reaction occurs in a laser wound in comparison to a scalpel wound. To explain this delay in epithelialization, they postulated that laser-induced thermal necrosis of the wound margin caused: 1) the formation of a firm eschar that impedes epithelialization and 2) the decreased contraction and scarring found with laser wounds increases the surface area to be epithelialized.[18] Moreno et al., using epidermal cell outgrowth from CO_2 laser cut explants, found that a delay in the onset of migration of the epithelium, not a decreased rate of migration, was responsible for the delayed epithelialization.[19] They speculated that the reduced inflammatory response retards the stimulus for epithelial migration by sealing the small vasculature and lymphatics and not allowing the release of chemical mediators.

The objectives of the present study were to document human responses to laser surgery evaluated by clinical and histologic evaluation. Specifically, we wanted to study whether de-epithelialization with the CO_2 laser of the outer gingival surface of a mucoperiosteal flap, at surgery and during the healing period, will retard epithelial migration and the formation of a long junctional epithelium.

MATERIALS AND METHODS

Six mandibular incisors, with a hopeless diagnosis demonstrated by probing depth and radiographic findings, were selected in 2 volunteers (a 43-year-old male and 60-year-old female). The protocol for this study was approved by the medical institutional research review board for human subjects at St. Mary Hospital Health Center. Both patients were in good health, received oral and written explanation of the protocol, and signed a detailed informed consent form.

Each patient received thorough oral hygiene instruction involving the modified Bass intrasulcular technique and the use of an interdental brush and floss. Scaling and root planing to reduce inflammation was completed prior to surgical therapy. Because of advanced mobility patterns, all teeth in the study were splinted on the lingual surfaces with an acid etched, light-cured composite material, prior to surgical procedures.

A split-mouth design was used, with similar defects on the experimental and control teeth. Contralateral teeth were randomly divided into a test and control group. The test site was de-epithelialized using the CO_2 laser at the time of flap surgery. Repeated de-epithelialization was performed at 10 day intervals for 30 days. The control site received a mucoperiosteal flap without laser treatment (Figs. 1, 2, and 3).

Figure 3. Reflection of full thickness flap exposing the shallow osseous defects on the test tooth (26).

Prior to initial surgery and block section, presurgical radiographs along with intraoral photographs were taken for documentation (Fig. 4). Clinical measurements were also taken with a calibrated periodontal probe to the nearest mm using the cemento-enamel junction (CEJ) as a fixed reference point. Measurements to the free gingival margin and probing depth were recorded prior to surgery and 1 to 2 weeks prior to block section, to measure gingival recession, pocket reduction, and clinical attachment level changes (Table 1).

Surgical Procedure

The patients were given block anesthesia using 2% xylocaine with 1:100,000 epinephrine and local infiltration with 1:50,000 epinephrine 2% xylocaine to control hemorrage. A reverse bevel incision was made extending one tooth laterally from the surgical site along with vertical releasing incisions one tooth mesial and distal to facilitate access. An effort was made at all sites at the time of the initial incision to remove the crevicular epithelium. Facial and lingual full thickness mucoperiosteal flaps were reflected. Following elevations of the flaps all granulation tissue coronal to the alveolar crest was removed to expose the root surfaces. The roots were then planed using hand instruments and a rotary carbide finishing bur. On the buccal aspect of each study tooth a reference notch was placed on the root at the height of the crest of the alveolar bone using a 1/2 round bur. The surgical site was irrigated with sterile saline and the flap margins were repositioned and sutured using 4-0 silk. The patients were placed on tetracycline 250 mg 4 times a day

Figure 4. Patient 1: Radiograph of the test tooth 26, prior to block section, 3 months following surgery (Note root notch).

for 7 days along with a 0.12% chlorohexidine gluconate rinse twice a day for 90 days.

The test site was treated in the same manner as the control except that prior to reflection the outer aspects of the mucoperiosteal flap from the free gingival margin to the mucogingival junction (both labial and lingual) were irradiated with the carbon dioxide laser.[‡] Following flap reflection, the same procedure was performed on the inner aspect of the flap. Using a power setting of 8 watts in a pulsed mode (repetition rate of 20 times per second with an exposure of 20 msec) and an 0.8 mm spot size in focus, all visible epithelium was removed from the outer and inner aspect of the flap. The resultant char layer was totally removed with moist gauze prior to replacing the flaps. Care was taken to avoid any laser contact to the root surface or the alveolar bone by placing a periosteal retractor between the hard and soft tissue and aiming the laser beam at a 90° angle to the soft tissue flap.

Postsurgery Treatment

Sutures were removed at 10 days postoperatively and the patients were instructed to use an extra soft toothbrush with

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[‡]Model LX-20 CO_2 laser, Luxar Corp., Bothell, WA.

Table 1. Clinical Response to the Use of the CO₂ Laser + Flap Debridement Versus Flap Debridement Alone

Patient	Tooth	Procedure	Preoperative PD (mm)	CEJ-BP (mm)	Postoperative PD (mm)	Recession (mm)	PAL (mm)
1							
Control site	23 M	OD	3	8	2	2	−1
	23 F	OD	4	7	1	1	+2
	23 D	OD	5	7	3	1	+1
Test site	26 M	OD + L	3	8	2	1	0
	26 F	OD + L	5	7	1	1	+3
	26 D	OD + L	5	7	2	1	+2
2							
Control site	23 M	OD	5	10	3	2	0
	23 F	OD	2	6	2	1	−1
	23 D	OD	3	6	2	1	0
Control site	24 M	OD	6	11	3	1	+2
	24 F	OD	5	8	3	1	+1
	24 D	OD	5	10	3	1	+1
Test site	25 M	OD + L	5	9	1	2	+2
	25 F	OD + L	4	10	2	2	0
	25 D	OD + L	2	8	2	1	−1
Test site	26 M	OD + L	2	8	1	2	−1
	26 F	OD + L	1	8	1	1	−1
	26 D	OD + L	3	9	2	1	0

PD = Probing depth.
CEJ-BP = attachment level.
PAL = gain or loss in clinical attachment level.
OD = open debridement.
OD + L = open debridement + laser.

the modified roll technique for 6 weeks and then were instructed to return to the modified Bass technique. On the experimental site at 10, 20, and 30 days the de-epithelialization procedure of the outer gingival surface only, as described previously, was repeated using topical anesthesia. The patients were then seen at 2-week intervals for 90 days for professional prophylaxis.

Ninety days following the surgical procedure, block sections which included gingival tissues, 2 mm of crestal alveolar bone, periodontal ligament, and a labial portion of the tooth were surgically removed. The block sections were then rinsed with sterile saline and fixed in 10% neutral buffered formalin.

Histological Preparation

At block removal, teeth were fixed in a 10% buffered formalin, decalcified in EDTA and embedded in paraffin. Step serial sections 8 μm thick were cut and stained for routine evaluation using hematoxylin-eosin and selective Mallory trichrome stains.

Clinical Findings

Both patients healed without complications. At 10 days post-operatively there appeared to be more rapid healing on all the control sites. At 60 days all sites appeared to be healed, with the exception of marginal inflammation on patient number two (at both test and control sites).

Clinical findings are summarized in Table 1. Presurgical probing depths were similar on the test and control sites on

both patients. However, attachment loss was greater on patient 2 than patient 1. Postoperative probing depths were reduced in both patients. Following surgery, regardless of treatment modality, gingival shrinkage/recession was present at all sites ranging from 1 to 2 mm. Change in attachment level ranged from −1 to +3 mm, with patient 1 showing the most significant gain on the test site. It should be noted that the number of sites are too small to produce data of statistical or clinical significance. However, histologic evaluations were performed to better document the healing response.

Histologic Observations

Control. In both patients the control specimens were similar, characterized by a long junctional epithelium extending the entire length of the notch (Fig. 5). Elongated rete pegs and limited inflammatory infiltrate were present in the underlying gingival connective tissue. Crestal resorption was obvious because of the distance observed between the alveolar crest and base of the notch. No evidence of osteogenesis was present at the alveolar surface. No evidence of cementogenesis was present in or apical to the notch (Fig. 6).

Experimental. The results at the test site varied between patients, and even at different sites in the same patient. In contrast to the control specimens, the test site in patient 1 was characterized by limited migration of junctional epithelium with the major portion of the notch filled with connective tissue (Fig. 7). Limited evidence of inflammatory
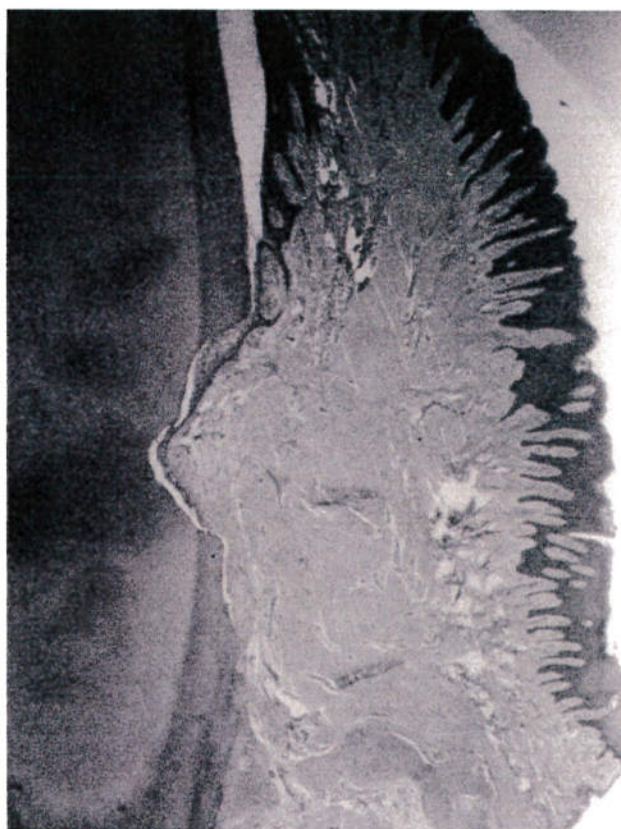
Figure 5. Control specimen (tooth 23): note long junctional epithelium continuing and occupying root notch. Crestal bone resorption is evident (original magnification × 12; H&E).

Figure 6. High power view of notch (Fig. 5) occupied by epithelium. No evidence of cementogenesis (original magnification × 28; H&E).

cells was present in the connective tissue opposite the notch. The inflammatory infiltrate was similar in the test and control specimens. The middle third of the root notch showed a connective tissue attachment to the root dentin. In the apical third of the notch, repair cementum was present (Fig. 8). On the surface of this cementum appears dense aggregates of active connective tissue cells with connective tissue fibers attached to the new repair cementum (Fig. 9).

Osteogenic activity appears to be present at both the crest and periodontal side of the alveolus. Osseous remodeling and reversal lines are present, but the lack of time-sequenced blocks precludes the knowledge of when bone apposition took place (Fig. 10). Resorptive root areas occurred apical to the notch and appear to be associated with active repair of both connective tissue and repair cementum (Fig. 11). There was no evidence of ankylosis in any of the sections.

The results at the test site in patient 2 were similar to the control with severe postsurgical gingival shrinkage, moderate to severe gingival inflammatory infiltrate, and the notch occupied by junctional epithelium.

DISCUSSION

The purpose of the present study was to evaluate whether de-epithelialization with a CO_2 laser at the time of flap surgery (with 3 follow-up treatments during the first post surgical month) would enhance the healing response of the open flap debridement procedure.

Observations indicate that in the laser-treated sites on patient 1, connective tissue and repair cementum formed in the root notch. This compared to a long junctional epithelial adhesion which occurred in all notches of the control teeth (flap debridement only) in the same patient.

The histologic results are consistent with Rossmann et al., in their 28-day monkey study.[16] Although that study evaluated interproximal intrabony defects, the CO_2 laser-treated sites, as compared to the control sites, indicated a greater amount of connective tissue attachment versus epithelium. In both studies the roots were notched at the alveolar crest. The present study showed the same trends in both individuals with all control notches fully repopulated with epithelium in the 90-day specimen and a shorter junctional epithelium present in the test site of both patients. However one again must note that there were too few cases to produce data of statistical or clinical significance.

The location of the root notch in the present study was at the alveolar crest, similar to Caffesse et al.[20] We utilized

Figure 7. Test tooth 26 with root notch occupied by connective tissue (original magnification × 13; H&E).
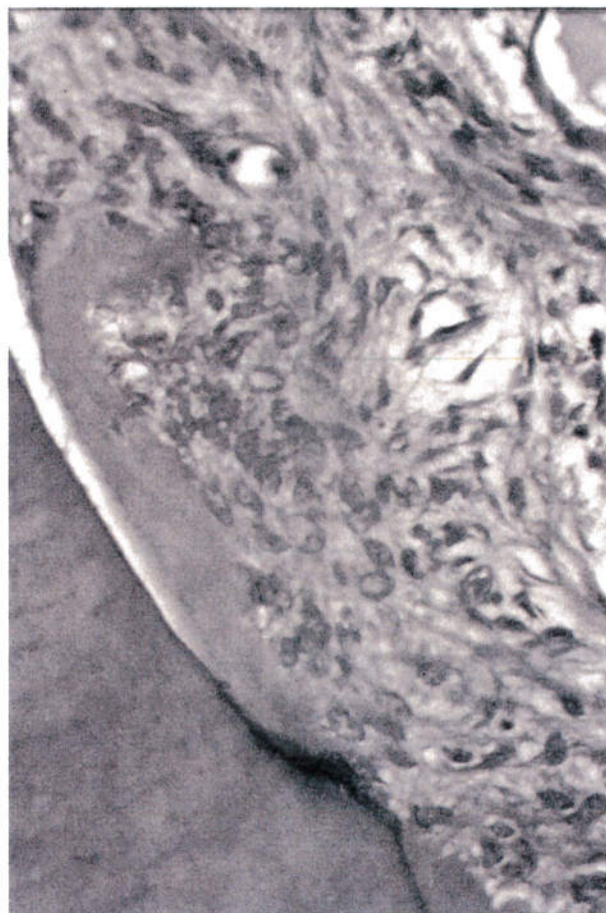

Figure 9. High power view of notch from Figure 7. Repair cementum formed on dentin with active connective tissue cells on cemental surface (original magnification × 112; H&E).

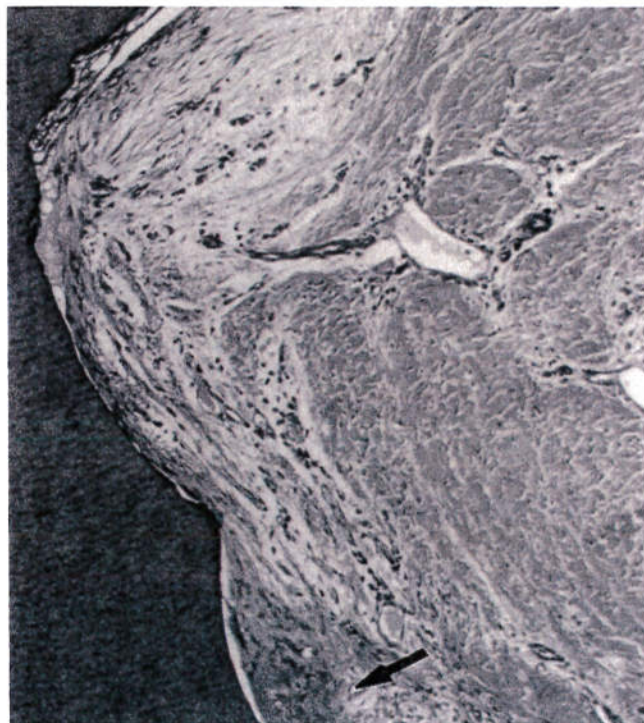*Figure 8. Note arrow indicating apical 1/3 of notch with repair cementum (original magnification × 28; H&E).*

this model rather than a calculus notch system[21] because of the limited subgingival calculus present on the treated teeth. The placement of the notch in this location, which had previously had supracrestal connective tissue attachment, precludes the possibility of determining if a new connective tissue attachment occurred on a root surface denuded of periodontal ligament fibers by periodontal disease. It appears that the control sites in both patients lost additional connective tissue attachment since epithelium was present in the notch. This healing response was also seen in only one of the two test patients. Since the results varied between patients and at different sites in the same patient, we must consider variations in human healing response in evaluating the results of this present study. Such variation in healing response has been seen using various bone replacement graft materials,[22-25] and membranes.[13,26,27]

Speculation as to why the notch in the laser-treated sites in patient 1 was repopulated with connective tissue and new cementum as opposed to the formation of epithelium in patient 2 may be in order. The presurgical gingival complex and keratinized tissue was of far less dimension in patient 2 than patient 1. This may have been responsible for greater
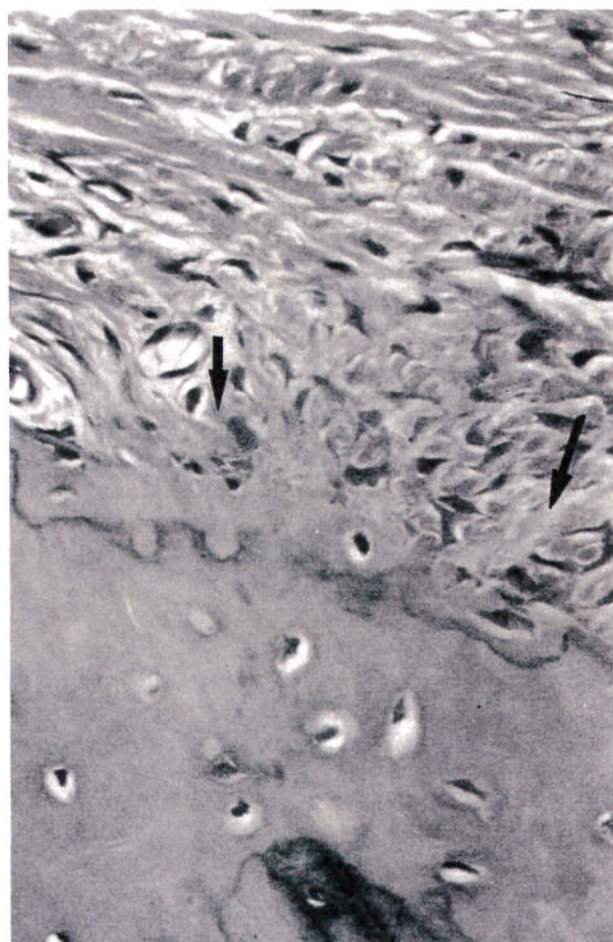
Figure 10. High power view of alveolar bone from Figure 7. Note arrows indicate areas with evidence of osseous remodeling at crest (original magnification × 112; H&E).

Figure 11. Higher power magnification of Figure 7 apical to root notch. Note repair of resorptive bays with connective tissue extending from bone to dentin and repair cementum at test site (original magnification × 112; H&E).

gingival recession and bone resorption in both the experimental and control sites of patient 2, thus limiting the healing potential of the periodontal ligament. Gottlow et al. have shown that healing utilizing guided tissue regeneration is limited by the amount of soft tissue coverage of the wound.[13] Stahl and Froum observed this limitation in their study using coronally anchored flaps.[28] Postsurgical plaque control was also a factor in the healed results. Both patients exhibited moderate to heavy levels of plaque at each follow-up visit. Poor compliance may also have been the result of the patient's knowing these teeth were to be lost.

This is the first human histological evaluation utilizing the CO_2 laser for de-epithelialization in evaluating postsurgical periodontal healing. On the basis of the reported observations, and based on these preliminary findings, further controlled follow-up studies may be warranted.

Acknowledgment

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