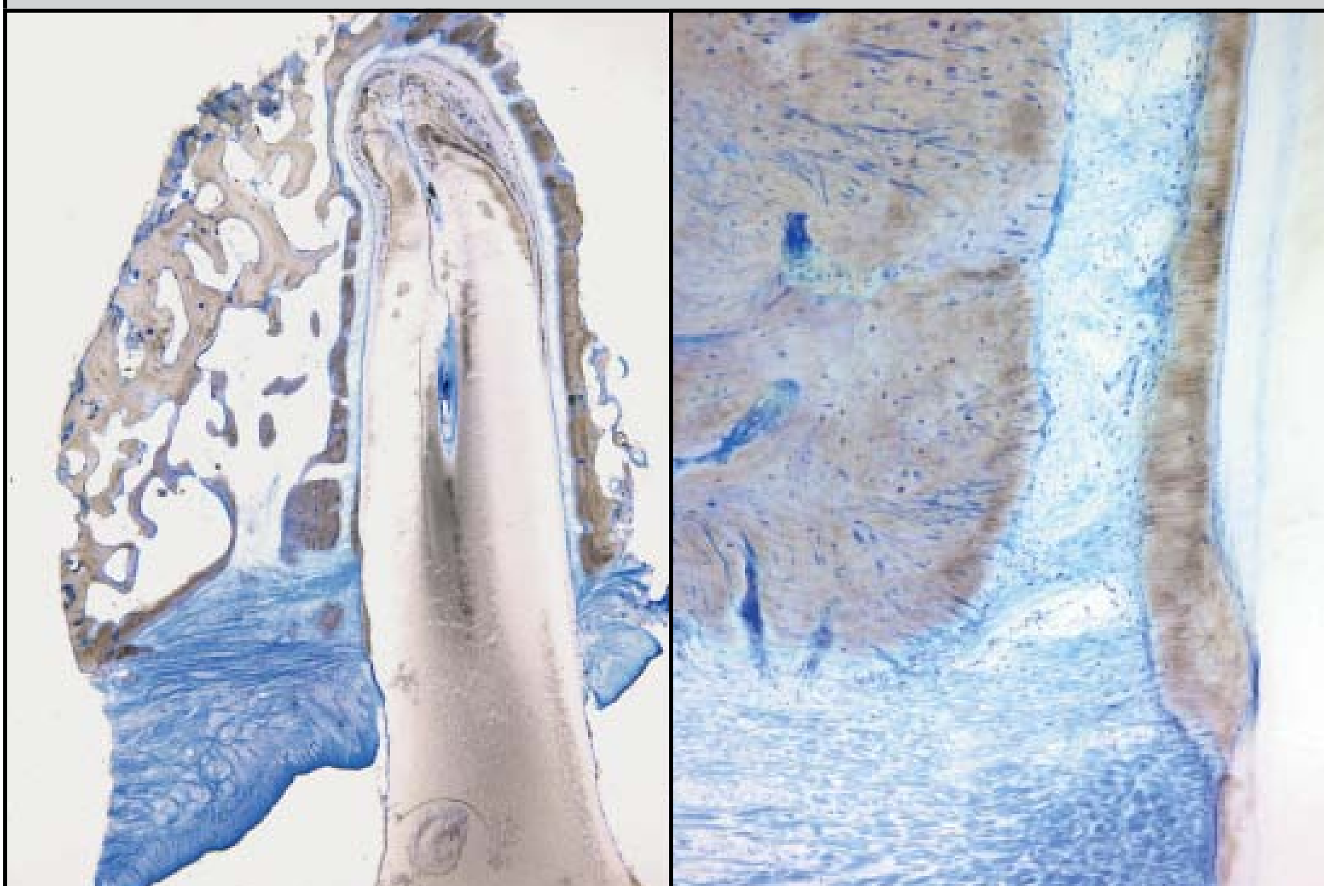

Human Clinical and Histologic Evaluation of Laser-Assisted New Attachment Procedure

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This investigation was designed to evaluate the healing response to the laser-assisted new attachment procedure (LANAP). Eight patients presenting with 12 teeth predetermined to be surgically extracted were enrolled and consented to treatment with full-mouth LANAP therapy. LANAP surgical therapy consisted of a first pass with a 360-µm fiber diameter, laser settings with verified output of 4.0 W and energy density of 1,965 mJ/mm^2, 100-µs pulse duration, and 20 Hz applied from the gingival margin to the base of the pocket parallel to the root surface and moved laterally and apically to remove the diseased pocket epithelium. The teeth were aggressively scaled and root planed with piezo ultrasonic instrumentation. A second pass was performed with a 360-µm fiber diameter, laser settings with verified output of 4.0 W and energy density of 1,965 mJ/mm^2, 650-µs pulse duration, and 20 Hz applied from the apical extent of the bone defect to the gingival margin. After 9 months of healing, en bloc biopsy extractions were provided. Ten teeth were analyzed histologically to assess the periodontal wound healing. Five teeth evidenced a degree of periodontal regeneration with new cementum, periodontal ligament, and alveolar bone. One tooth had new attachment with new cementum and inserting collagen fibers, and four teeth healed via a long junctional epithelium. LANAP therapy should be further investigated with long-term clinical trials to compare the stability of clinical results to conventional therapy. This report provides evidence that LANAP therapy can induce periodontal regeneration. (Int J Periodontics Restorative Dent 2012;32:497–507.)

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Laser therapy remains controversial in the field of periodontics.[1–3] Lasers of varying wavelengths (635 to 10,600 nm) used for nonsurgical and surgical periodontal and peri-implant therapy include neodymium:yttrium-aluminum-garnet (Nd:YAG), carbon dioxide, diode, and erbium:yttrium-aluminum-garnet (Er:YAG).[4–7] It is important to note that lasers of varying wavelengths have different levels of tissue penetration depending on reflection, scatter, and absorption.[8] Therefore, each therapy must be individually investigated with a specific laser. Each laser cannot be expected to replicate results of a laser of a different wavelength even when used to perform a similar therapy. Periodontal therapy utilizing a laser has been reported as a monotherapy,[9] as an adjunct to scaling and root planing,[5] for root debridement combined with surgical or nonsurgical therapy,[9,10] and to perform surgical laser-assisted new attachment procedures (LANAP).[11,12]

The primary goal of periodontal therapy is to establish periodontal health with pocket reduction and attachment gain, preferably through periodontal regeneration.[13] Periodontal regeneration is defined as establishing a new attachment apparatus on a previously diseased root surface via new cementum, new periodontal ligament (PDL), and new bone.[13] Multiple regenerative therapies have demonstrated adequate documentation of new periodontal structures regenerated adjacent to a calculus notch to meet these criteria.[13–19] These therapies all utilize surgical placement of a bone replacement graft material (autogenous, allogenic, or xenogenic) or a biologic agent (growth factor or amelogenins) with or without the combination of a barrier membrane. Most of these reports include the use of periodontal surgical flap procedures. Mellonig et al[20] reported the ability to stimulate periodontal regeneration when combining scaling and root planing with enamel matrix derivative application as a nonsurgical/flapless therapy.

There is desire among clinicians and patients to identify less invasive therapeutic options that can provide periodontal regeneration. Given the variations in clinical predictability of current therapies, it is unusual to provide full-mouth therapy via regenerative techniques even though patients may present with periodontal disease throughout their dentition. There is an undertreatment of periodontal disease based on patient and clinician perceptions regarding negative side effects of periodontal flap surgery such as pain, recession, dentinal sensitivity, and postoperative discomfort.[12] Minimally invasive laser periodontal therapy utilizing the patented LANAP protocol has been advocated for periodontal treatment but has limited clinical research demonstrating its efficacy and predictability. There is little known about the biology of the wound-healing process for these procedures.

The purpose of this study was to histologically evaluate the healing response to minimally invasive laser-assisted periodontal surgical therapy (LANAP).

Method and materials

This investigation was designed and implemented as a single-center, prospective clinical study with enrollment completed from May 1 through June 30, 2010. Subjects provided informed consent according to the Declaration of Helsinki of 1975, as revised in 2000. A total of 12 periodontal defects were identified in 8 subjects with advanced periodontitis. Eligibility required subjects to be between the ages of 18 and 70 years and have at least 1 tooth diagnosed and prescribed for extraction with a probing depth ≥ 7 mm and radiographic suggestion of a 4-mm or deeper intrabony defect. Subjects were excluded from the study if they had congenital or metabolic bone disorders, immunosuppressive therapy, or disease that may affect wound healing; were current smokers (within 6 months of entry into the study); or were pregnant or planning pregnancy during the course of the study. In addition, subjects with a history of active periodontal surgical treatment or scaling and root planing during the past 12 months were excluded.

At the screening visit, eligibility was assessed, patient histories were taken, and extra- and intraoral examinations were performed. The study teeth and primary study sites were identified prior to beginning the surgical visit, and baseline clinical measurements were recorded. Full-mouth LANAP therapy was provided in a single visit one arch at a time.

Clinical measurement and notch validation

Prior to enrolling patients, the periodontal examiner participated in a calibration training session. Probing and recession depth were measured and attachment levels calculated. Levels of intra- and interexaminer reliability were found to be within prespecified limits, with greater than 90% repeatability.

A notch validation study was performed prior to initiating study procedures. Six teeth requiring extraction from patients not enrolled in the LANAP protocol were included after consent was obtained for participation. Prior to extraction, the level of calculus was identified with tactile sensation using a periodontal probe and no. 23 dental explorer. The site was then notched with a no. 1/2 round bur utilizing high-speed instrumentation at its apical extent that could be accessed with a dental handpiece. No flap was raised. The notch was placed on the root surface with a horizontal groove into the cementum through the calculus. The teeth were extracted, photographed, and placed in formalin. Evidence of calculus apical to the notch was then confirmed via light microscopy and scanning electron microscopy. The calculus extended below the notch with a mean of 2.23 ± 1.28 mm, demonstrating a diseased root surface apical to the level of clinically detected and notched calculus.

Surgical procedure

The minimally invasive laser periodontal surgical therapy was provided using an Nd:YAG laser (PerioLase MVP-7, Millennium Dental Technologies). The surgical procedure was as follows: Clinical measurements for probing depth, recession depth, mobility, and furcation grade were accomplished for the study teeth according to the calibration protocol prior to administering local anesthesia. A notch was placed at the estimated apical extent of calculus on the study teeth using a no. 1/2 round bur with high-speed instrumentation to place a horizontal groove at the identified level on the root, and the location of the notch was measured relative to the cementoenamel junction for future reference. The laser was first used at a setting of 4.0 W, 100-μs pulse duration, and 20 Hz. It was passed from the gingival margin to the base of the pocket parallel to the root surface and moved laterally and apically to remove the diseased pocket epithelium and decontaminate the pocket. This was accomplished for all surfaces of the pocket for teeth in a single arch. The teeth were aggressively scaled and root planed with piezo ultrasonic instrumentation. Four or five piezo tips (Piezon Master 400, EMS and Piezosurgery, Mectron) were used on each root surface with repetitive cleaning until the roots were smooth and there was no visual or tactile evidence of remaining calculus. This can result in reduction of the depth of the previously placed notch, necessitating another measurement to confirm the notch location. The root preparation was performed from the coronal aspect of the teeth to the base of the intrabony defect, including the notched root surface. Once root preparation was complete, the circumferences of the teeth were passed with hand instrumentation to the level of the PDL either with a Piezo tip insert (EMS) or with a no. 11 periodontal knife. An attempt was made to bluntly dissect any remaining fibers and to press the instrument into the PDL space to stimulate vascular access to the periodontal wound. A second pass with the laser at a setting of 4.0 W, 650-μs pulse duration, and 20 Hz was performed from the apical extent of the defect to the gingival margin. The surgical sequence was repeated for the other arch.

Occlusal adjustment with selective grinding and extra- or intracoronal splinting of the teeth was provided immediately postoperatively and as needed at follow-up visits to eliminate mobility. Patients were given the following postoperative instructions and medications: 0.12% chlorhexidine mouthrinse bid for 4 weeks, oral antibiotics (amoxicillin 500 mg) every 8 hours for 7 days, and anti-inflammatory analgesics for pain relief as needed (ibuprofen 600 mg, every 6 hours). Tooth-brushing was modified to protect against dislodging the fibrin clot. Patients were instructed to only brush the coronal tooth surfaces for the first 2 weeks postoperatively.

Patients were seen for follow-up care and oral hygiene instructions at 7, 14, 28, 42, and 56 days. The surgical sites were inspected and gently cleaned with chlorhexidine-soaked gauze, and teeth were gently cleaned supragingivally. Prophylaxis was provided, and hygiene was reviewed with the patient at 2.5, 4, 5.5, 7, and 8.5 months. Radiographs were updated at 5.5 and 9 months.

At 9 months, en bloc biopsy of the study teeth was performed. Prior to providing local anesthesia, full-mouth clinical measurements were performed, including probing depth, recession depth, mobility, and furcation grade. Guiding grooves for histologic preparation were placed on the coronal tooth structure over the study site. Each study tooth and a measured amount of surrounding tissue and bone were removed en bloc as described previously.[17] Biopsy sites were reconstructed with regenerative procedures in preparation for implant placement and subsequent prosthetic reconstruction with implant-supported prostheses. Biopsy specimens were stored in 10% formalin.

Microcomputed tomography

Specimens were scanned using a high-resolution microcomputed tomography (micro-CT) system (µCT 40, Scanco Medical) in multislice mode. Each image dataset consisted of approximately 600 micro-CT slice images. The specimens were scanned in high-resolution mode with an x-, y-, and z-resolution of 16 µm. The image datasets were used to produce three-dimensional views of the specimens using special software (Scanco Medical).

Light microscopy

Fixed samples were prepared for the preparation of nondemineralized ground sections according to the technique of Donath and Breuner.[21] The core specimens were processed by dehydration in a graded series of alcohols over a period of at least 9 days at standard temperature and pressure while constantly shaking. Then, the specimens were infiltrated with a graded series of alcohols and Technovit 7200 VLC embedding resin (Heraeus Kulzer) over a period of at least 12 days at standard temperature and pressure while constantly shaking. When finished, specimens were placed in three consecutive containers of 100% Technovit 7200 VLC for 24 hours each at standard temperature and pressure while constantly shaking.

Following dehydration and infiltration, specimens were embedded in Technovit 7200 VLC and polymerized using 450 nm of light for 10 hours, never exceeding 40°C.

Polymerized blocks were sliced longitudinally along the orientation grooves of the teeth, applied by the surgeon using an Exakt cutting unit (Exakt). This involved preparing a section of approximately 150 µm using the cutting/grinding instrument and then finishing the section to 30 to 50 µm using the microgrinding unit. A final polish was used with 0.1-µm diamond polishing paste.

The sections were stained with Sanderson RBS (methylene blue and potassium permanganate stain). Sections were enclosed by cover slips for analysis by means of both brightfield and polarized light microscopic evaluation.

Backscatter scanning electron microscopy

Specimens were glued to aluminum holders for backscatter scanning electron microscopy evaluation. The surface to be examined was highly polished with diamond pastes and thoroughly cleaned in an ultrasonic cleaner. Thereafter, the polished surface was sputter-coated with a 6-nm-thick carbon layer using an SCD-500 sputter coater (Bal-Tec). The specimen was examined using

Table 1 Clinical results (mm) for study teeth at baseline and 9 months

Patient	Tooth no.*	Baseline			9 mo			PD reduction	CAL gain
		PD	Recession	CAL	PD	Recession	CAL		
1	34	9	0	9	3	4	7	6	2
2	41	8	2	10	3	5	8	5	2
2	45	14	2	16	4	5	9	10	7
3	34	10	0	11	7	4	8	3	3
3	33	10	0	10	5	2	7	5	3
4	15	10	2	12	8	2	10	2	2
5	36	16	0	16	6	1	7	10	9
5	33	12	0	12	4	3	7	8	5
5	45	9	0	9	6	1	7	3	2
6	26	9	2	11	4	2	6	5	5
7	46	8	0	8	4	3	7	4	1
8	27	10	0	10	6	0	6	4	4
Mean		10.4	0.7	11.2	5.0	2.7	7.4	5.4	3.8
Standard deviation		2.43	0.98	2.55	1.60	1.61	1.16	2.64	2.38

PD = probing depth; CAL = clinical attachment level.
*FDI tooth-numbering system.

a Zeiss VPN-40 field cathode scanning electron microscope using the backscatter detector.

Results

Eight patients with 12 teeth identified for extraction and histologic analysis were enrolled in the study. All sites healed uneventfully with no unexpected adverse events related to the LANAP surgical treatment. The mean total energy used per patient was 6,034 J, with a mean 3,344 J for the mandible and 2,691 J for the maxilla. Some patients experienced increased dentinal sensitivity during the first 4 weeks postoperatively, which then decreased to within normal limits. There were no signs of root damage from the laser therapy noted clinically or histologically. The 12 study teeth presented with a mean initial clinical attachment level (CAL) of 11.2 ± 2.50 mm, probing depth (PD) of 10.4 ± 2.43 mm, and recession of 0.7 ± 0.98 mm. After 9 months, these sites had a mean CAL gain of 3.8 ± 2.38 mm and PD reduction of 5.4 ± 2.64 mm. This resulted in a postoperative mean CAL of 7.4 ± 1.16 mm, PD of 5.0 ± 1.60 mm, and recession of 2.7 ± 1.61 mm (Table 1).

Table 2 Histologic analysis of study teeth

Patient (tooth no.*)	Healing mechanism
1 (34)	Regeneration
2 (41)	LJE
2 (45)	New attachment
3 (34)	LJE
3 (33)	LJE
4 (15)	LJE
5 (36)	Regeneration
5 (33)	Regeneration
6 (26)	Regeneration
8 (27)	Regeneration

LJE = long junctional epithelium.
*FDI tooth-numbering system.

Twelve teeth were evaluated using micro-CT and then prepared for histologic evaluation. Two teeth splintered during histologic preparation and were not available for microscopic evaluation. Periodontal wound healing evaluation coronal to the calculus notch revealed that five teeth healed with periodontal regeneration, evidencing new cementum, new PDL, and new alveolar bone (Table 2). One tooth had a new attachment apparatus with new cementum and inserting collagen fibers, and four teeth healed via junctional epithelium.

Four teeth in three patients healed via long junctional epithelium, with one of these evidencing new cementum and reattachment apical to the calculus notch. Two teeth (patient 3, mandibular left canine and first premolar) that healed with a long junctional epithelium are presented as a case example. Neither the postoperative periapical radiograph nor the micro-CT scan evidenced any bone fill of the intrabony defects (Figs 1a to 1c). Histologic assessment revealed healing via long junctional epithelium to the level of the calculus notch (Figs 1d and 1e), with no new cementum or new bone.

Periodontal regeneration coronal to the calculus notch was confirmed for three teeth presenting with furcation involvement. One of these teeth presented with a grade III furcation (patient 6, maxillary left first molar), and two teeth were identified clinically as grade II

Fig 1a *Pretreatment periapical radiograph demonstrating intrabony defects.*

Fig 1b *Posttreatment radiograph showing persistence of the defects.*

Fig 1c *Micro-CT scan confirming persistence of the defects.*

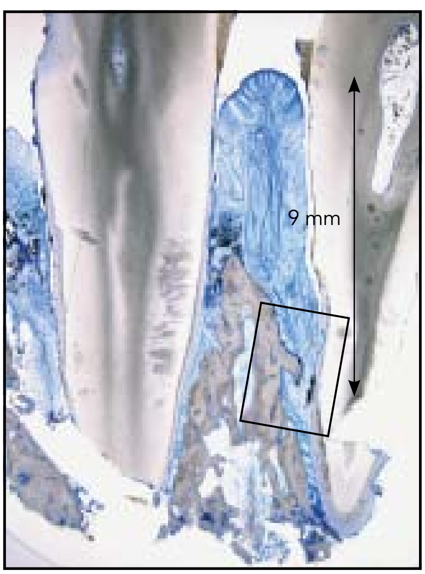
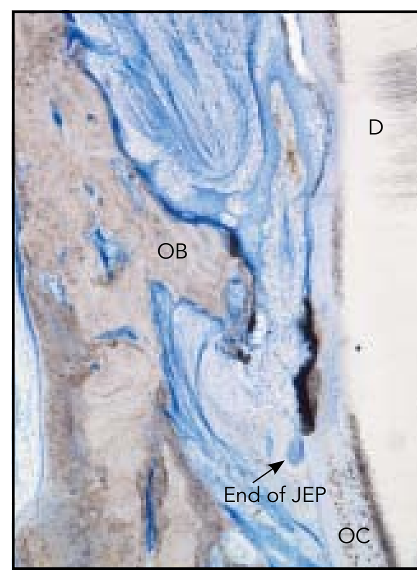


Fig 1d (left) *Panoramic histologic view used to assess the healing and the notch measurement to confirm the notch location. Healing was noted via long junctional epithelium.*

Fig 1e (right) *Higher magnification reveals the long junctional epithelium extending to the level of the calculus notch. OB = old bone; D = dentin; JEP = junctional epithelium; OC = old cementum.*



furcations (patient 3, mandibular left first molar and patient 8, maxillary left second molar) but surgically identified as grade III furcations. Although none of the furcations closed or were reduced in grade, they demonstrated significant CAL gain and histologic assessment of regeneration.

The mandibular left first premolar in patient 1 presented with a 9-mm CAL and 9-mm PD at baseline. After 9 months, there was a 2-mm CAL gain with a 4-mm recession and residual probing depth of 3 mm. The micro-CT scan easily identified the level of the calculus notch and was used in establishing

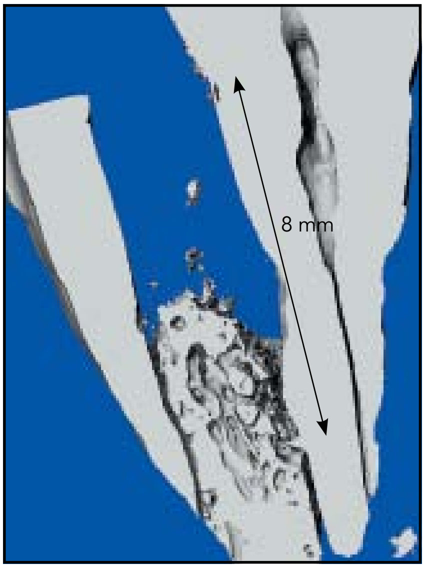


Fig 2a (left) *Micro-CT scan with 8-mm notch measurement from the cemento-enamel junction. There appeared to be significant bone fill of the defect proximal to the root surface.*

Fig 2b (right) *Histology was consistent with the micro-CT scan. New cementum* (NC), *periodontal ligament* (PDL), *and new bone* (NB) *were evident, demonstrating periodontal regeneration. The epithelium was limited coronal to the regeneration* (end of JEP). *Note the cervical notching of the root surface from the extensive root planing to remove the heavy calculus deposits. OB = old bone; JEP = junctional epithelium.*

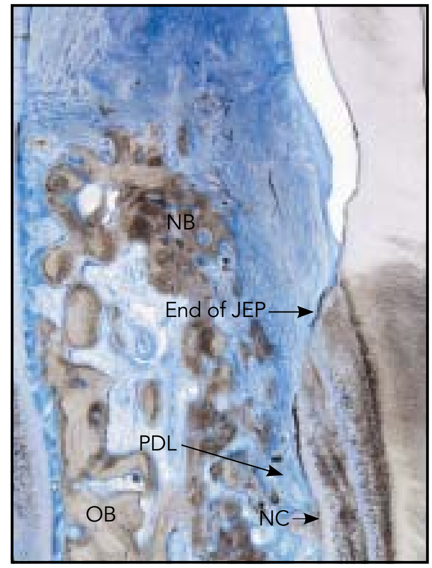


Fig 3a *Maxillary left first molar presenting with an initial CAL of 11 mm, PD of 9 mm, and grade III furcation.*

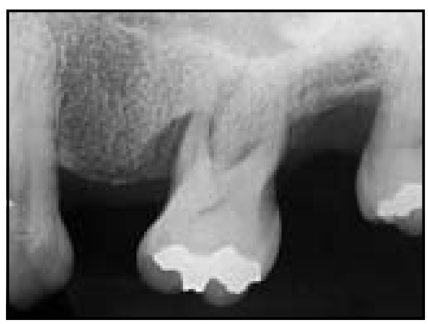


Fig 3b *Preoperative radiograph demonstrating a deep intrabony defect on the mesial surface and suggesting furcation invasion.*

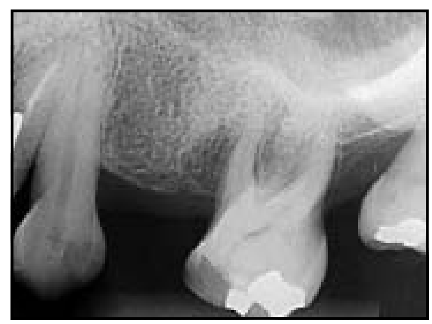


Fig 3c *Postoperative radiograph suggestive of bone fill and improved prognosis.*

the histologic landmarks (Figs 2a and 2b). There was an outgrowth of new cementum at the level of the calculus notch, which extended coronally with the adjacent PDL and new alveolar bone (Fig 2b).

A maxillary left first molar (patient 6) presented with a deep intrabony defect on the mesial surface with a baseline CAL of 11 mm and PD of 9 mm (Figs 3a and 3b). The postoperative radiograph showed bone fill (Fig 3c) and a 5-mm CAL gain with no recession (postoperative measurements: CAL, 6 mm; PD, 4 mm). Histologic evaluation revealed new bone proximal to the mesial root surface (Fig 3d). The higher-magnification view revealed a thick layer of new cementum on the root surface extending several millimeters coronal to the calculus notch with inserting Sharpey fibers crossing the PDL to the adjacent

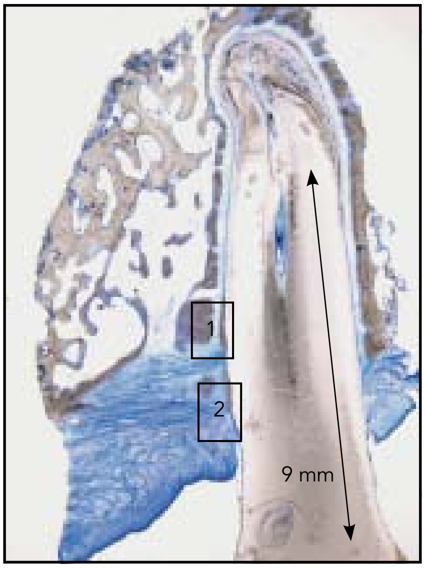


Fig 3d *Panoramic view showing bone fill of the intrabony defect and periodontal regeneration. The 9-mm notch measurement from the cementoenamel junction confirms the notch location.*

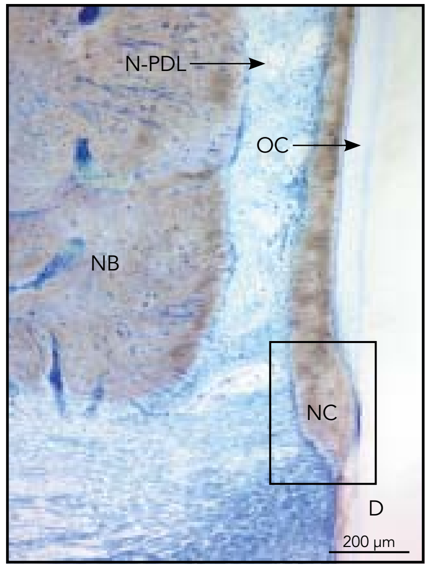


Fig 3e *Higher-magnification view of box 1 in Fig 3d revealed a layer of new cementum* (NC) *extending to the coronal extent of the defect with adjacent periodontal ligament* (N-PDL) *and new bone* (NB) *defining periodontal regeneration. OC = old cementum; D = dentin.*

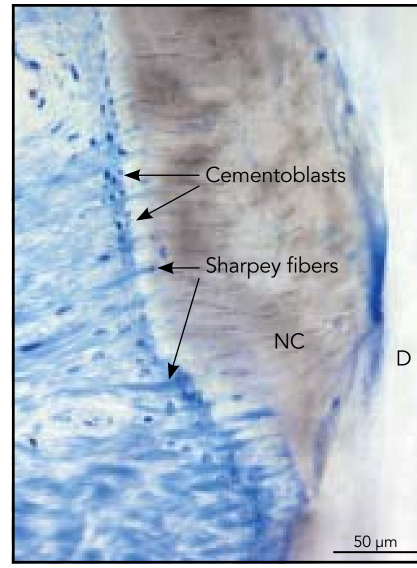


Fig 3f *Inserting Sharpey fibers into the new cementum and presence of cementoblasts (magnified view of box in Fig 3e). NC = new cementum; D = dentin.*

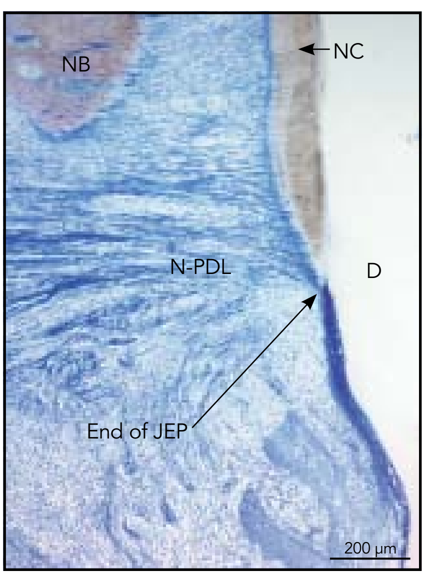
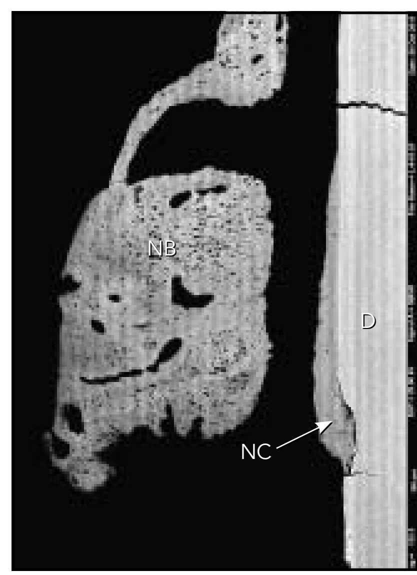


Fig 3g (left) *The supracrestal environment regenerated to its natural state with supracrestal inserting collagen fibers into the new cementum* (NC) *just apical to the junctional epithelium* (JEP) *(higher-magnification view of box 2 in Fig 3d). NB = new bone; N-PDL = new periodontal ligament; D = dentin.*

Fig 3h (right) *Backscatter electron microscopy confirmed the histologic assessment. The layers of root surface with dentin* (D), *old cementum, and new cementum* (NC) *could be well visualized. NB = new bone.*



alveolar bone (Fig 3e). Cementoblasts were visible on the surface of the cementum (Figs 3f and 3g). Backscatter electron microscopy confirmed the findings from the light microscopic examination and helped to visualize the layers of root structure, including dentin, old cementum, and new cementum (Fig 3h).

Discussion

This report presents evidence that using LANAP therapy for microinvasive periodontal therapy may result in periodontal regeneration adjacent to a calculus notch placed on the previously diseased root surface. Six of 10 teeth evaluated demonstrated a regenerative response, with 5 undergoing periodontal regeneration and 1 receiving new attachment. The ability to improve the periodontal condition even for sites with furcation involvement was evident, with 3 teeth with grade III furcation involvement demonstrating some degree of periodontal regeneration.

The only previous histologic report on the LANAP protocol was that published by Yukna et al,[22] in which six LANAP-treated teeth demonstrated evidence of new attachment with new cementum and inserting collagen fibers. Teeth treated with scaling and root planing healed via long junctional epithelium, with one site evidencing minimal new cemetum. There are differences in the treatment protocols that should be highlighted, including that this report allowed 9 months of healing versus only 3 months for the study by Yukna et al.[22] The current study utilized full-mouth LANAP treatment, whereas the previous study utilized a split-mouth design, treating the specific study teeth either with laser surgery or scaling and root planing, and limited the root preparation coronal to the calculus notch. This study provided root preparation with intensive root planing with piezo ultrasonic instrumentation from the coronal aspect of the teeth down to the level of the PDL. Of interest is that the previous histologic study demonstrated new cementum and new attachment even though there was no root preparation below the notch where there may have been additional toxins or calculus. Previous publications have demonstrated evidence of human histology for regeneration with new cementum over old cementum, dentin, calculus, and an enamel projection.[14,19]

Validation for notch placement was necessary because the previously accepted definition for notch placement is that of a visual procedure. The validation confirmed that tactile sensation can be used to place the notch in calculus with a no. 1/2 round bur and high-speed instrumentation in a flapless environment preoperatively. The use of a notch measurement and the micro-CT scan were helpful to confirm the location of the notch for histologic assessment.

LANAP appears to be a safe procedure that resulted in apparent periodontal regeneration. There were no significant side effects beyond dentinal hypersensitivity or gingival recession and no damage to the root surfaces.[23] Without histology, it would not be possible to evaluate the biologic potential of the surgical procedure, and this information is essential to combine clinical and radiographic assessment and the healing potential for patient care.

Recent clinical innovations and research have focused on minimally invasive surgical therapies.[24–27] For example, Cortellini and Tonetti demonstrated improved wound stability using a modified minimally invasive surgical technique (M-MIST) that increased the likelihood of blood clot stabilization as a principle of periodontal wound healing for regeneration.[28] This research was further supported by a prospective study comparing M-MIST with and without application of enamel matrix derivative.[29] The outcomes were similar for both groups, with CAL gains of 4.1 ± 1.4 mm for the M-MIST control group and 4.1 ± 1.2 mm for the EMD/M-MIST group. The improved wound stability appears to be directly related to the periodontal wound-healing results. This is similar with the LANAP procedure, which utilizes a microinvasive approach. After the first laser pass, the tissues are relaxed and can be gently reflected to visualize the root surface with the use of limited magnification (4.5× for this study). The second pass with the laser stimulates the blood clot, which is the foundation for periodontal wound healing.

Conclusions

This study provides further evidence that periodontal regeneration may take place following treatment via LANAP. It should be noted that an apical calculus notch may not have been placed in the exact manner as required by the definition for periodontal regeneration.

Acknowledgment

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