Histologic Evaluation of an Nd:YAG Laser–Assisted New Attachment Procedure in Humans

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This report presents histologic results in humans following a laser-assisted new attachment procedure (LANAP) for the treatment of periodontal pockets. Six pairs of single-rooted teeth with moderate to advanced chronic periodontitis associated with subgingival calculus deposits were treated. A bur notch was placed within the pocket at the clinically and radiographically measured apical extent of calculus. All teeth were scaled and root planed with ultrasonic and hand scalers. One of each pair of teeth received treatment of the inner pocket wall with a free-running pulsed neodymium:yttrium-aluminum-garnet (Nd:YAG) laser to remove the pocket epithelium, and the test pockets were lased a second time to seal the pocket. After 3 months, all treated teeth were removed en bloc for histologic processing. LANAP-treated teeth exhibited greater probing depth reductions and clinical probing attachment level gains than the control teeth. All LANAP-treated specimens showed new cementum and new connective tissue attachment in and occasionally coronal to the notch, whereas five of the six control teeth had a long junctional epithelium with no evidence of new attachment or regeneration. There was no evidence of any adverse histologic changes around the LANAP specimens. These cases support the concept that LANAP can be associated with cementum-mediated new connective tissue attachment and apparent periodontal regeneration of diseased root surfaces in humans. (Int J Periodontics Restorative Dent 2007;27:577–587.)

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Regeneration of the supporting tissues of the teeth is a primary goal of periodontal therapy. Whereas clinical results and animal histology suggest that new connective tissue attachment (CTA) as well as regeneration of cementum (CEM), periodontal ligament (PDL), and alveolar bone (AB) can occur on human teeth affected by periodontitis as a result of several treatment approaches, histologic evidence in humans of successful cases and successful treatments is limited.[1]

The 1996 World Workshop in Periodontics established specific histologic criteria for proof of regeneration. Experimental teeth must have loss of CTA and AB associated with periodontitis. In addition, subgingival and/or subcrestal calculus must be present at the time of surgery so that a notch can be made into the root at the apical extent of calculus. Proof of new attachment is demonstrated by new CEM and CTA, and regeneration is evidenced by the presence of new CEM, PDL, and AB coronal to the apical extent of the notch. Most treatments that show proof of new attachment and regeneration are associated with surgically implanted devices or materials.[1–21]

Sulcular/pocket epithelium removal has been the basis or foundation of subgingival curettage (CUR), the excisional new attachment procedure (ENAP), and the replaced flap/modified Widman flap procedure to set up an environment for new CTA.[22–25] However, elimination of pocket epithelium by CUR, ENAP, or other internal-bevel incision designs appears nearly impossible.[26]

Procedures limited to treating the soft tissue wall of periodontal pockets such as CUR and ENAP would not be expected to influence new bone formation to any great degree but hopefully would lead to healing with a CTA rather than a long junctional epithelium (LJE). Almost all available human histologic evidence to date demonstrates healing by an LJE with no or minimal CTA.[27]

Interest in neodymium:yttrium-aluminum-garnet (Nd:YAG) laser use in periodontics is increasing. Several papers have suggested favorable results with its use in the treatment of periodontal pockets.[28–30] A procedure called laser ENAP has been promoted in trade journals with examples of radiographic bone regeneration.[31,32] Referred to as the laser-assisted new attachment procedure (LANAP) in this report, this technique of pocket therapy has recently been approved by the US Food and Drug Administration (FDA 510k clearance K030290).

In clinical case reports LANAP has demonstrated improved clinical measurements and some radiographic evidence of bone regeneration in the areas treated.[33–37] However, it is not known what tissues constitute the new healed interface between the soft tissues and the tooth root. Also, there is some evidence that the use of lasers in periodontal pockets may damage root surfaces,[38–49] adversely affect the adjacent alveolar bone,[50,51] or cause undesirable pulpal changes.[48,49] Clinical case reports have reported favorable results, but there is no human histologic proof of the nature of the healing following LANAP. The purpose of this paper is to report histologic wound healing following use of LANAP surgery for periodontal pockets.

Method and materials

Dental radiographs of patients assigned to the Postgraduate Periodontics Clinic, Louisiana State University (LSU), were screened for the presence of teeth that had isolated moderate to severe periodontal involvement (probing depths and clinical probing attachment loss of 5 to 9 mm with bleeding on probing and evident subgingival calculus). Teeth that had been treatment planned by clinicians in the Oral Diagnosis and/or Prosthodontics departments for extraction as part of the overall restorative treatment plan were included in the study. Subjects had to provide two single-rooted teeth with similar periodontal involvement for the study and signed an LSU-approved consent form prior to beginning the study.

Preoperatively, the subjects received occlusal adjustment/odontoplasty to reduce occlusal forces on the experimental teeth, and study teeth were splinted to neighboring teeth with an extracoronal bonded splint (Ribbond, Ribbond Inc). Scaling and root planing were performed on other teeth in the same segment (not the treatment teeth), and general supragingival prophylaxis was provided for the rest of the mouth.

Documentation consisted of clinical photographs, radiographs with stent and grid (Fig 1), modified Gingival Index (mGI),[52] Quigley-Hein Plaque Index (PI),[53] and clinical mobility evaluation.[54] Clinical measurements were made from the cementoenamel junction (CEJ) to the free gingival margin, from the CEJ to the base of the pocket, from the CEJ to the apical extent of clinically and radiographically evident calculus, and from the CEJ to the mucogingival junction. Bleeding on probing (BOP) was also assessed.

Appropriate laser safety precautions were used. Under regional local anesthesia, a quarter-round bur notch was placed at the clinically and radiographically measured apical extent of calculus as carefully as possible. One of each pair of teeth randomly received Nd:YAG laser treatment (Periolase, Millennium Dental Technologies) of the inner pocket wall to remove the crevicular epithelium around the necks of the study teeth, relax the gingival collar, and expose more of the contaminated root surface. The fiber tip of the laser was directed parallel to the root surface and was moved laterally and apically along the pocket wall, eventually reaching close to the base of the pocket. The laser settings for this first pass were 3 W, 150-μs pulse duration, and 20 Hz. Once the epithelial lining was removed, root debridement was accomplished coronal to the area of the calculus reference notch with ultra-

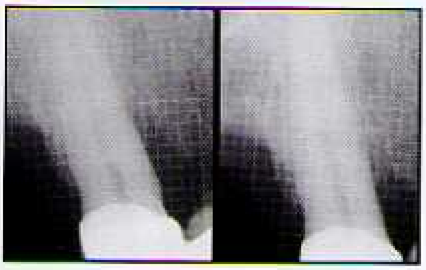
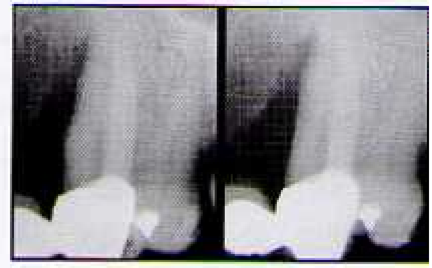


Fig 1 *Radiographs of a 54-year-old man with deep infrabony defects on the mesial of both maxillary canines.*

Fig 1a *(left) Mesial defect on the right canine (left), treated with LANAP, demonstrates a radiographic increase in bone density and apparent fill of defect at 3 months after treatment (right).*

Fig 1b *(right) Mesial defect on the left canine (left), treated with scaling and root planing without laser, demonstrates little change in the bony defect contour or bone density after 3 months (right).*



sonic (EMS Piezon 400, EMS) and hand instrumentation. No attempt was made to remove any soft/granulation tissue with the mechanical instrumentation. The pocket contents of the test teeth were lased again (4 W, 635-µs pulse duration, and 20 Hz) to help achieve a solid fibrin clot and form a pocket seal. The control teeth received all of the aforementioned treatment except for the laser therapy. No sutures were used, and triple antibiotic ointment and a light-cured dressing (Barricaid, Dentsply/Caulk) were placed on all teeth. All patients were provided with nonsteroidal anti-inflammatory medications, doxycycline (100 mg daily for 10 days), and 0.12% chlorhexidine rinses (to be used twice daily).[55]

After 3 months, a second surgical procedure was performed to remove the experimental tooth roots en bloc according to methods described previously.[4,9,56,57] For all teeth this was a single proximal area. The body of each tooth root was bisected longitudinally in a faciolingual plane, with the clinician attempting to keep at least half of the root diameter attached to the area of interest. A small interproximal wedge of tissue and a section of root approximately 5 mm wide, 7 mm long, and 5 mm thick was removed. Once the desired specimens were completely freed, they were gently and atraumatically removed, rinsed gently in sterile saline, and placed in 10% neutral buffered formalin. The residual defects were reconstructed, and after an appropriate healing period, the patients were referred for prosthetic replacements.

The biopsy specimens were processed by the LSU School of Dentistry Research Histology Laboratory, where they were decalcified, embedded in paraffin so as to obtain longitudinal mesiodistal serial step sections, serially sectioned at 7 µm in the area of the notch, and stained with hematoxylin and eosin. The three most central 200-µm serial step sections were blindly and randomly evaluated for the nature of the healed tissues—specifically the presence and length of new CEM, new CTA, new AB, and healed junctional epithelium relative to the apical extent of the calculus notch. Histomorphometric measurements were made by an oral pathologist (RLC) using an eyepiece grid on the microscope. Root resorption, ankylosis, pulpal changes (where pulp tissue was visible), and the degree of inflammation were also evaluated. Mean values for the three sections of each tooth were used for linear measurements.

Results

Three men and three women, 26 to 54 years old (mean 45.5 years), provided two teeth each. All subjects tolerated the treatment procedures well and reported that almost no pain-relieving medication was needed after the laser treatment. All teeth healed uneventfully. The LANAP-treated teeth exhibited greater mean probing depth reduction (4.7 mm vs 3.7 mm) and greater clinical probing attachment level gain (4.2 mm vs 2.4) than the con-

Table 1 Clinical changes (3-month mean results, in millimeters) following use of LANAP or scaling and root planing alone (n = 6 teeth for each treatment)

Measurement/treatment	Pretreatment	3 mo	Change
Gingival recession			
LANAP	0.2*	0.1*	0.2
SCL/RP	0.3*	0.6	0.8
Probing depth			
LANAP	7.3	2.7	4.7
SCL/RP	8.0	4.3	3.7
Vertical CAL			
LANAP	7.2	3.0	4.2
SCL/RP	7.6	5.3	2.4

LANAP = laser-assisted new attachment procedure; SCL/RP = scaling and root planing; CAL = clinical attachment level.
*Coronal to cementoenamel junction.

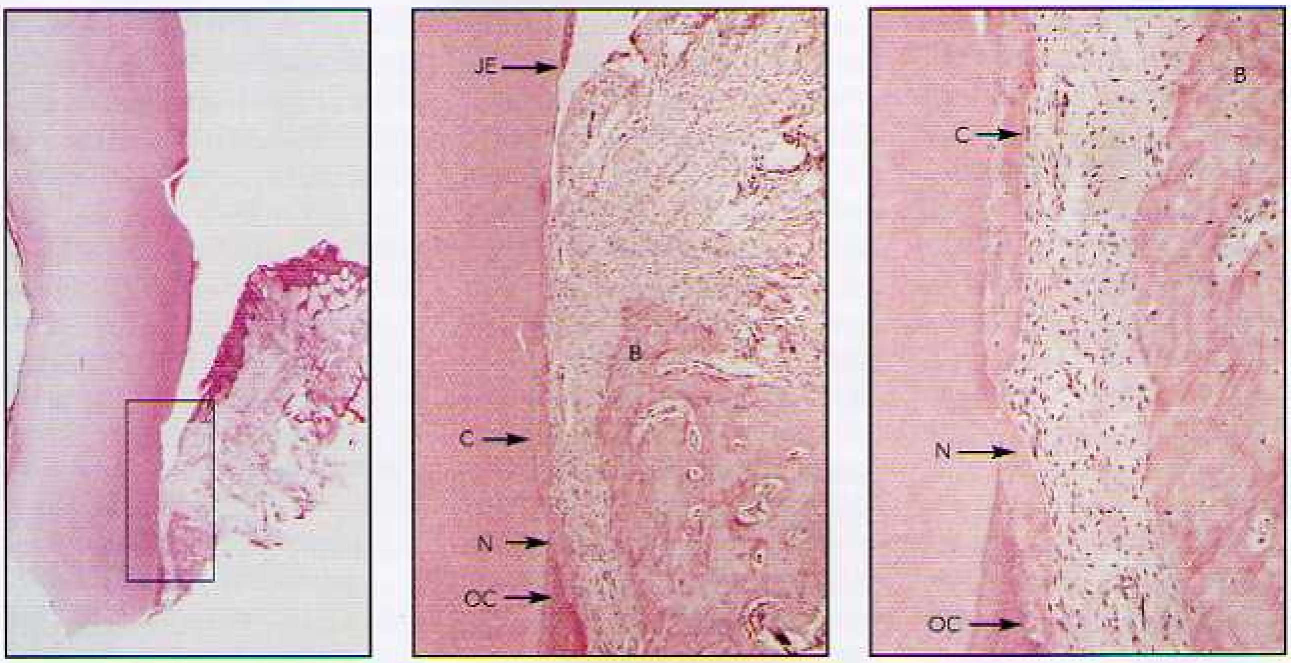


Fig 2 *Histologic views of LANAP-treated maxillary right canine from Fig 1a (hematoxylin & eosin). (left) Low-power view (×1) with box around area of interest. (center) Medium-power view (×16) showing calculus notch (N) with new cementum (C) in and coronal to the notch and old cementum (OC) apical to the notch, apical extent of junctional epithelium (JE), and new bone (B) adjacent to the notch. (right) High-power view (×40) of notch area demonstrating new cementum (C) filling the notch (N) and extending coronally, old cementum apical to the notch (OC) covered by new cementum, new alveolar bone (B), and new periodontal ligament and gingival fibers attached to the tooth.*

trol teeth. Clinical results are presented in Table 1. mGI, PI, and BOP were improved on all test and control teeth. Total energy applied to the test pockets ranged from 14 to 25 J/mm of probing depth (mean 19 J/mm).

All six LANAP-treated specimens showed new CEM and new CTA in and occasionally coronal to the notch (Figs 2 to 5). In two specimens, the notch was within the infrabony pocket (subcrestal) and the new CEM and new

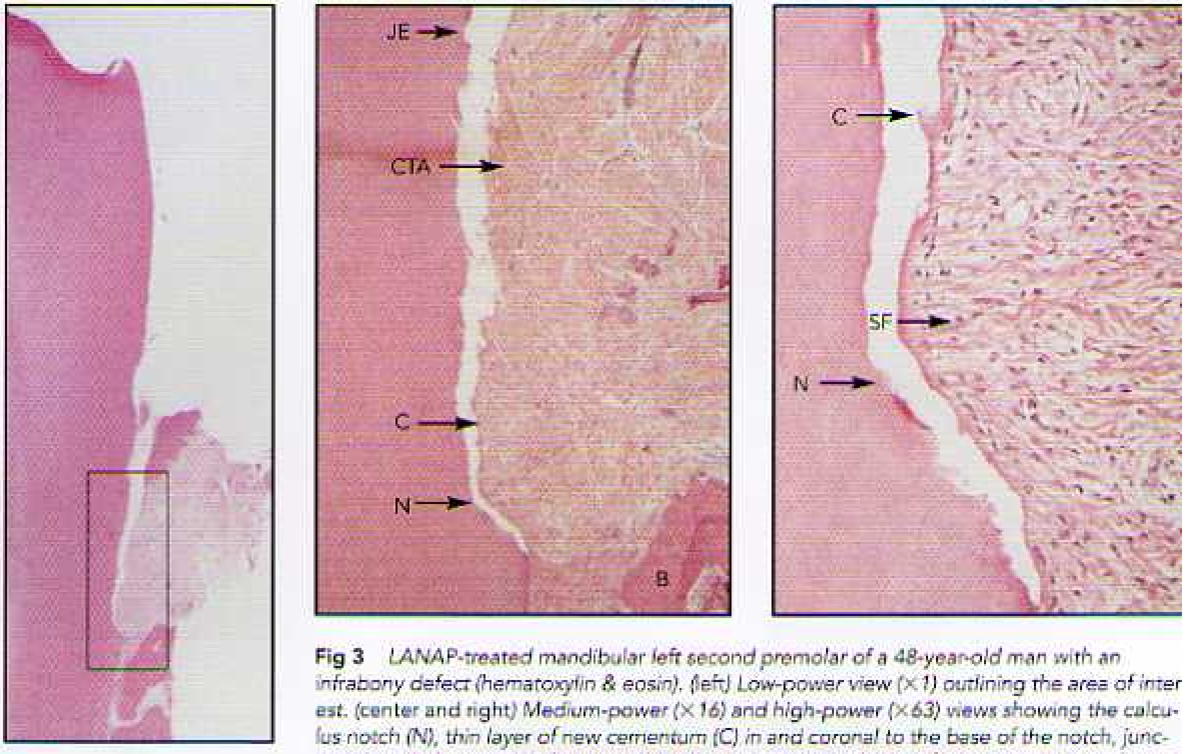


Fig 3 *LANAP-treated mandibular left second premolar of a 48-year-old man with an infrabony defect (hematoxylin & eosin). (left) Low-power view (×1) outlining the area of interest. (center and right) Medium-power (×16) and high-power (×63) views showing the calculus notch (N), thin layer of new cementum (C) in and coronal to the base of the notch, junctional epithelium (JE) at the coronal level, new CTA with Sharpey fibers (SF), and new bone (B) adjacent to the notch. (Cementum is artificially separated from tooth.)*

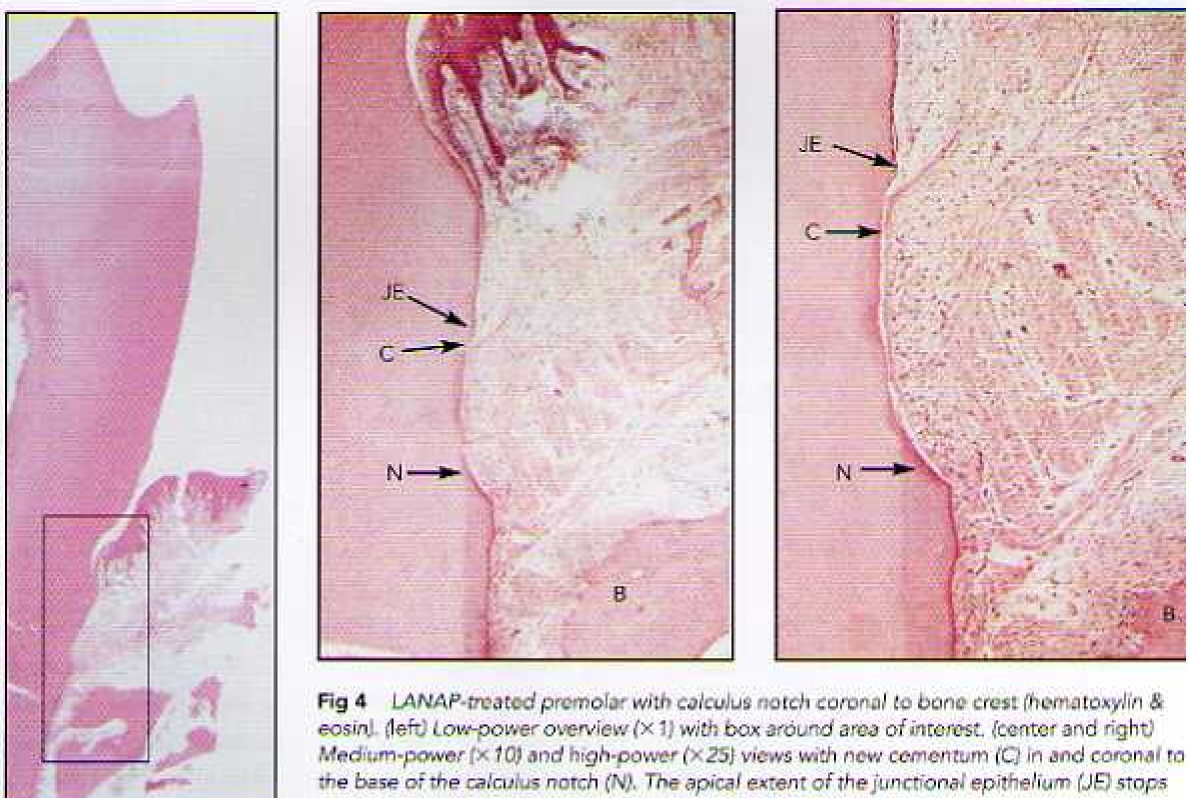


Fig 4 *LANAP-treated premolar with calculus notch coronal to bone crest (hematoxylin & eosin). (left) Low-power overview (×1) with box around area of interest. (center and right) Medium-power (×10) and high-power (×25) views with new cementum (C) in and coronal to the base of the calculus notch (N). The apical extent of the junctional epithelium (JE) stops near the coronal limit of new cementum. B = alveolar bone.*

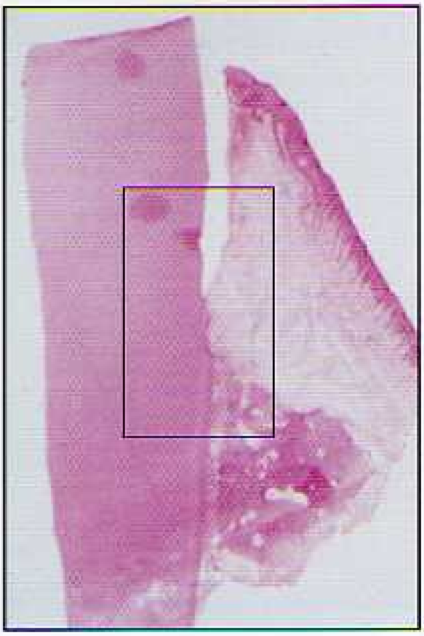


Fig 5 Canine tooth with calculus notch coronal to bone crest treated with LANAP (hematoxylin & eosin). (left) Low-power (× 1) view with box around area of interest. (right) Medium-power (× 10) view showing new cementum (C) in and coronal to the calculus notch (N). New CTA is evident between new cementum and the apical extent of the junctional epithelium (JE).

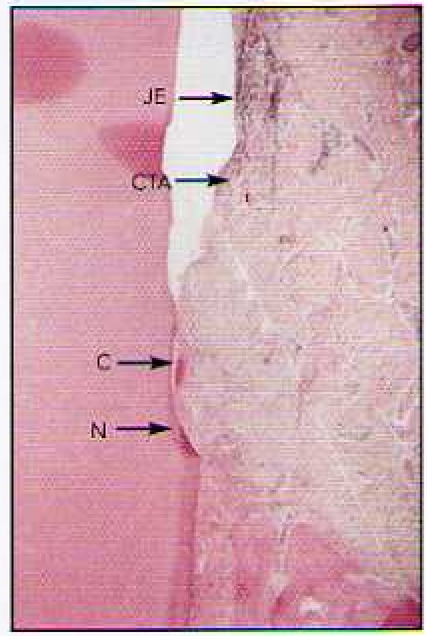


Table 2 Frequency of histologic findings following the use of LANAP or scaling and root planing alone (3-month results, n = 6 teeth for each treatment)

Measurement/Treatment	Frequency
New cementum	
LANAP	6/6 (1.2*)
SCL/RP	1/6 (0.1*)
New bone	
LANAP	4/6
SCL/RP	2/6
New CTA	
LANAP	6/6
SCL/RP	1/6

*Mean amount, in millimeters, from micrometer readings.
LANAP = laser-assisted new attachment procedure; SCL/RP = scaling and root planing; CTA = connective tissue attachment.

CTA were adjacent to new AB, technically showing periodontal regeneration. Five of the six control teeth had an LJE, with no evidence of new attachment or regeneration (Figs 6 and 7). One control specimen did show a small amount (0.1 mm) of new CEM and CTA. There was no evidence of any adverse histologic changes to the root surface or the pulp of any of the teeth. Histologic results are presented in Table 2.

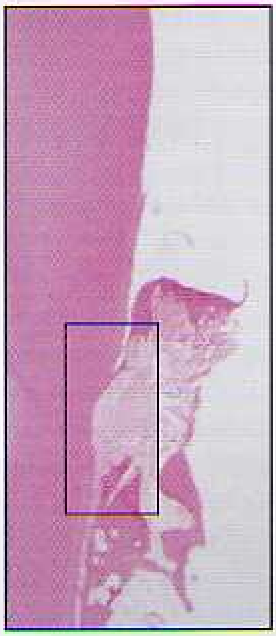
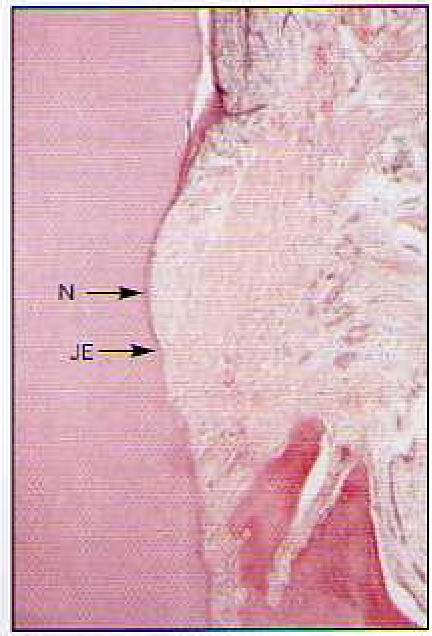


Fig 6 Control tooth treated with scaling and root planing without laser application (hematoxylin & eosin). (left) Low-power (× 1) view with box around area of interest. (right) Medium-power (× 10) image showing calculus notch (N) with no evidence of new CEM, new AB, or new CTA. The junctional epithelium (JE) extends to the apical extent of the notch.



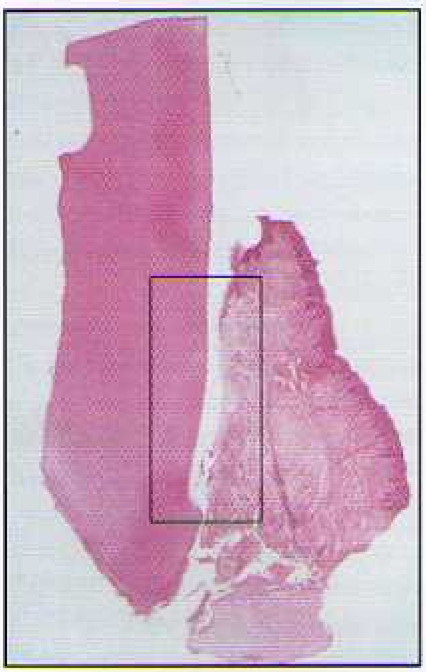
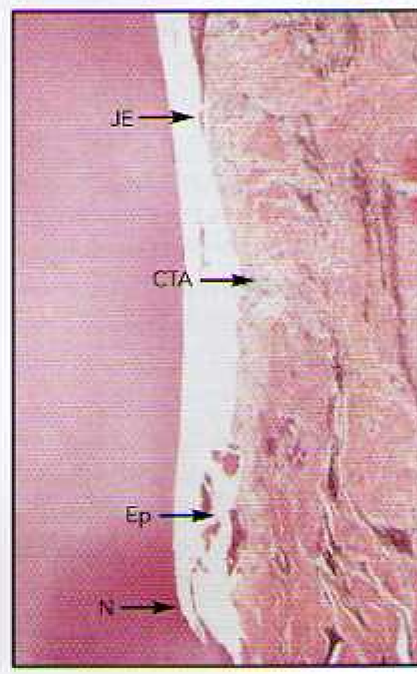


Fig 7 Mandibular premolar that received control treatment (scaling and root planing alone) (hematoxylin & eosin). (left) Low-power (× 1) view with box around area of interest. (right) Medium-power (× 10) view demonstrating lack of good tissue contact with root, even though CTA is present between the calculus notch (N) and junctional epithelium (JE). No new CEM is evident, and some epithelial islands (Ep) are present at the depth of the pocket and as islands within the connective tissue.



Discussion

This human histologic report demonstrates favorable histologic healing with the use of the free-running pulsed Nd:YAG laser used in a specific patented technique of LANAP. Apparent periodontal regeneration (CEM, PDL, AB) on a calculus- and plaque-contaminated area of the root was seen on two of the test teeth, and CEM-mediated new attachment was evident on the other four laser-treated teeth. Similar periodontal healing in humans has been shown with other surgical techniques.[2–21,58–60]

The histologic assessment was based primarily on presence/ absence criteria but also included linear measurements of new CEM length. As with the control teeth in this report, the literature demonstrates consistent and almost universal healing by LJE following scaling and root planing, gingival curettage, and open flap debridement procedures[2,3,56,57,59–62] and variable histologic results with bone replacement graft materials and miscellaneous regenerative agents on contaminated root surfaces.[3–21,59,60,61,63–68]

Comparison of the results of this study with those from a study that used demineralized freeze-dried bone allografts[2] suggests essentially equivalent histologic results using the LANAP procedure. In this study new CEM was seen in 100% of the cases versus 77% of the cases in the Bowers et al[2] study; new CEM length was the same (1.2 mm) as in Bowers et al; and the frequency of new CTA was 100% versus 68% for Bowers et al. It should be noted that the number of specimens was larger in the study of Bowers et al.

Treatment allocation could not be concealed from the therapist, as he had to use the laser on one tooth and not the other. Treatment (laser or no laser) was allocated according to a random code after all preliminary measurements and procedures, including placement of the calculus notch, had been completed.

Accurate placement and evaluation of the calculus notch presented several challenges. Since no flaps were reflected, direct visualization of the calculus was not possible. Positioning of the notch was based on repeated measurements from the CEJ to the clinically detectable calculus and evaluation of calculus when it was evident on the radiographs. The appropriate "depth" was marked on the shank of the quarter-round bur, and the notch was placed as carefully as possible. Again, because no flap was reflected, the depth of the notch into the root was confined to the lateral part of the bur head and was necessarily limited. It is true that there is no direct way to guarantee that the notch was actually placed in calculus, but this was the most tedious and difficult part of the entire procedure because the depth of the bur placement and therefore the depth of the notch was based on clinical and/or radiographic detection of calculus. Since clinical and radiographic detection of calculus leads to many false-negative but no false-positive results, it is felt that this was as accurate as could be accomplished with the closed procedure employed. In addition, if any error was made it was to place the notch more coronally to be sure that it was in calculus and/or contaminated root surface. On the histologic slides, the position of the notch was verified by using the clinical measurements related to the CEJ or biopsy-related landmarks.

It should be emphasized that the LANAP is a combined therapy using a patented protocol (US patent #5,642,997) that includes several aspects: occlusal adjustment, splinting when needed, systemic and topical antibiotics, laser use for surgical pocket epithelium removal, scaling and root debridement, and laser use for tissue stabilization (welding) against the tooth surface with a fibrin clot. Use of the laser without attention to these other aspects may not yield the results reported here. It should be recognized that LANAP is a single-treatment surgical procedure. Since tissue is surgically removed from the lining of the pocket with the laser (rather than with a scalpel) and occlusal adjustment is an integral part of the protocol, it would appear that only qualified clinicians can legally perform the treatments in most locales.

In conclusion, this study demonstrated consistently positive histologic responses in periodontal pockets in humans treated with the LANAP. CEM-mediated new attachment and occasionally apparent periodontal regeneration following a specific protocol with a free-running pulsed Nd:YAG laser were demonstrated.

Acknowledgments

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