Immediate Effects of Laser-Assisted New Attachment Procedure (LANAP) on Human Periodontitis Microbiota

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Abstract

Objective: The Laser-Assisted New Attachment Procedure (LANAP) surgical protocol was compared to ultrasonic root debridement alone for immediate post-treatment effects on putative bacterial pathogens in deep human periodontal pockets.

Methods: In a case series of 26 systemically-healthy adults with severe periodontitis, 20 patients were treated with the LANAP surgical protocol and 6 patients received ultrasonic root debridement alone. LANAP surgery was performed using a free-running, pulsed Nd:YAG laser, with laser energy (4.0 W, 150-µs pulse duration, 20-Hz) first directed circumferentially around teeth parallel to root surfaces in a coronal-apical pass to probing depth for selective pocket epithelium ablation and to initiate reflection of a gingival flap. After ultrasonic root debridement and gingival flap advancement to the alveolar bone crest, a second laser pass (4.0 W, 650-µs pulse duration, 20-Hz) was similarly performed in an apical-coronal direction to thermally induce a fibrin clot at the tooth-gingival flap interface. Subgingival biofilm specimens were collected before and immediately after completion of the treatments from 2 inflamed periodontal sites with ≥ 6 mm probing depths on a single tooth per patient, and selected periodontal pathogens identified using established anaerobic culture techniques.

Results: Red and orange complex bacterial species were culture-negative immediately post-treatment in 17 (85%) of 20 LANAP-treated patients, but only 1 (16.7%) of 6 patients subjected to ultrasonic root debridement alone.

Conclusions: The LANAP surgical treatment protocol, but not conventional ultrasonic root debridement alone, immediately suppressed red and orange complex periodontal pathogens below culture detection limits in most deep human periodontal pockets.

Key words: Periodontitis, laser(s), microbiology, non-surgical periodontal therapy, periodontal surgery

Introduction

The Laser-Assisted New Attachment Procedure (LANAP) is a minimally invasive periodontal surgery protocol that employs a neodymium-doped yttrium aluminum garnet (Nd:YAG) laser (Gregg and McCarthy, 2001; Mizutani *et al.*, 2016). Favorable clinical and radiographic changes have been reported after LANAP surgery on severe periodontitis lesions (Harris *et al.*, 2004; Harris *et al.*, 2014; Yukna *et al.*, 2007; Tilt, 2012; Nevins *et al.*, 2012; Nevins *et al.*, 2014; Brown, 2013). Histologically, LANAP surgery may induce periodontal regeneration on human root surfaces, where a new functionally-oriented periodontal attachment apparatus, comprised of new cementum and alveolar bone with inserting periodontal ligament fibers, develops in the apical portions of periodontitis lesions (Yukna *et al.*, 2007; Nevins *et al.*, 2012). In a 3-month post-LANAP surgery histologic study of 6 periodontitis-affected teeth, 2 teeth revealed evidence of periodontal regeneration, 4 teeth developed new periodontal attachment (i.e., new cementum with inserting periodontal ligament fibers), and no teeth healed with only a long junctional epithelium

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(Yukna *et al.*, 2007). In a separate 9-month post-LANAP surgery histologic study on 10 periodontitis-affected teeth, periodontal regeneration occurred on portions of 5 (50%) teeth, new periodontal attachment on 1 tooth, and long junctional epithelium healing alone on 4 teeth (Nevins *et al.*, 2012). A systematic review on treatment of periodontal intrabony defects from the 2014 American Academy of Periodontology Regeneration Workshop found, in regard to the LANAP surgical protocol, that "despite the evidence for new attachment and periodontal regeneration, information about clinical predictability of this procedure has yet to be demonstrated", but suggested that "because of the minimally invasive nature and expendable surgical materials required, this approach may be appropriate for multiple [intrabony] defects as a first line of management" (Kao *et al.*, 2015). In this regard, the United States Food and Drug Administration, based on safety and effectiveness data provided by the manufacturer, expanded the oropharyngeal indications for use of the Nd:YAG laser employed in LANAP surgery in 2016 for which it is cleared for marketing in the United States to include "periodontal regeneration – true regeneration of the attachment apparatus (new cementum, new periodontal ligament and new alveolar bone) on a previously diseased root surface when used specifically in the LANAP® Protocol" (available on-line at https://www.accessdata.fda.gov/ cdrh_docs/ pdf15/K151763.pdf ; Suzuki and Sullivan, 2017).

One important determinant in the success or failure of periodontal regeneration surgical therapy is the composition of subgingival plaque biofilms on treated tooth surfaces (Nowzari *et al.*, 1995). High total bacterial counts and large numbers of red cluster complex species, *Aggregatibacter actinomycetemcomitans*, *Prevotella intermedia/ nigrescens*, *Parvimonas micra* and/or *Staphylococcus aureus*, in subgingival biofilms on treated tooth surfaces at the time of guided tissue regeneration surgery and during post-treatment healing have been negatively correlated to successful therapeutic outcomes (Machtei *et al.*, 1994; Nowzari *et al.*, 1995; Heitz-Mayfield *et al.*, 2006).

The antimicrobial effects of LANAP surgery on subgingival biofilm populations, which may contribute to favorable treatment outcomes, have not been evaluated to date. This report assessed the immediate post-treatment effects of the LANAP surgical protocol, as compared to ultrasonic root debridement alone, on putative bacterial pathogens in deep human periodontal pockets.

Materials and methods

Patients

A patient case series of 26 adults with untreated localized to generalized Stage III/Grade B periodontitis (severe) (Tonetti *et al.*, 2018) were identified in a private periodontal specialty practice in Ft. Lauderdale, FL, USA, when being examined for periodontal disease-related concerns. Subgingival microbial specimens were collected from the patients in 2009, prior to the conceptualization of the present retrospective data analysis, before and immediately after completion of initial periodontal therapy by the treating periodontist to help determine the potential need for and selection of systemic antimicrobial therapy. The 26 patients were in good systemic health, had at least 22 teeth, had not received any systemic antibiotic therapy within the 3 months prior to pre-treatment examinations, and yielded pre-treatment subgingival microbial specimens with cultivable periodontal pathogens at elevated levels (Rams *et al.*, 1996). Persons with aggressive periodontitis, gingival pain, acute periodontal conditions, a history of diabetes mellitus, venereal disease, blood dyscrasias, or anomalies of the immune system, or who required prophylactic antibiotics for dental treatment, were pregnant, or had received immunosuppressive drug therapy within the previous 6 months were excluded from the patient case series. The patients were subjected without randomization to either the LANAP surgical protocol (n = 20 patients, 10 male, 10 female, mean age 56.1 ± 9.1 (standard deviation; SD) years, age range 39 - 74 years), or ultrasonic root debridement alone (N = 6 patients, 3 male, 3 female, mean 47.3 ± 15.5 (SD) years, age range 26 - 70 years), as they were identified and consented to treatment.

The periodontal examinations, treatment procedures, and subgingival microbial biofilm sampling were conducted with informed consent of the patients in compliance with the Helsinki Declaration of 1975, as revised in 2000. Approval for this retrospective analysis of the patient case series data was provided by the Temple University Human Research Protection Program Institutional Review Board. No additional patient consent was required, because the retrospective analysis involved evaluation of preexisting data coded from archived dental records without unique patient identifiers, and no further patient contact.

Periodontal Treatments

Patients receiving the LANAP surgical protocol were treated without any pre-surgical initial periodontal therapy (Mizutani *et al.*, 2016). In brief, a free-running, pulsed, 1,064 nm wavelength-specific Nd:YAG laser (Periolase MVP-7, Millennium Dental Technologies, Inc., Cerritos, CA, USA), with an attached 360 μm diameter optical glass fiber held in a metal handpiece with a flexible tip, was set at a 20-Hertz repetition rate, 4.0 Watts average power, and a 150 fμs pulse duration, providing an energy density of 196 Joules/cm^2, a power density of 3,930 Watts/cm^2, and a peak power of 1,333 Watts/pulse. The optical glass fiber was cleaved prior to each clinical use per manufacturer's instructions; an internal power meter was used to verify the laser's power output to within 0.1 Watts; and an internal

Joule counter tabulated laser light doses applied to patients. Laser wavelength-specific protective eyewear was utilized by all dental personnel and patients during Nd:YAG laser treatment. The exposed end of the laser optical fiber, emitting a red aiming beam and a light energy dose of 200 milli-Joules/pulse, was initially advanced circumferentially around all teeth parallel to root surfaces in a coronal-apical pass to probing depth for selective pocket epithelium ablation (Gold and Vilardi, 1994; Ting *et al.*, 2014), and to initiate reflection of a gingival flap without vertical releasing incisions (Mizutani *et al.*, 2016), delivering a dose of 10-12 Joules per mm of probing depth at each periodontal site. This was followed by ultrasonic root debridement for 60 seconds per tooth with a P1 scaler tip on a piezoelectric ultrasonic scaler (Piezon® Master 400, Electro Medical Systems SA, Nyon, Switzerland) set at 75% power level, and using a 0.03% chlorhexidine solution made from a 4-fold dilution of a 0.12% chlorhexidine gluconate mouthrinse (Peridex™ Oral Rinse, 3M ESPE Dental Products, St. Paul, MN, USA) for irrigation and cooling. Ultrasonic root debridement aimed to remove microbial biofilms and dental calculus from tooth surfaces without deliberate removal of root cementum (Laleman *et al.*, 2017). After ultrasonic root debridement, the gingival flap was advanced to the alveolar bone crest by blunt dissection to induce bleeding into the pocket, and to physically modify the surface of the osseous crest to promote a regional acceleratory phenomenon at the healing site (Verna, 2016). A second laser pass was then carried out in an apical-coronal direction with the Nd:YAG laser set at a 20-Hertz repetition rate, 4.0 Watts average power, and a 650 μs pulse duration, providing a peak power of 308 Watts/pulse. This delivered 4 to 5 Joules per mm of probing depth at treated sites to thermally induce a fibrin clot at the tooth-gingival flap interface for hemostasis and soft tissue adhesion to the tooth (Mizutani *et al.*, 2016). The total energy dose of 14 to 17 Joules per mm of probing depth applied to each treated periodontal pocket as a result of both Nd:YAG laser passes was within a recognized desired and safe range for human periodontal sites (Harris, 2003). No sutures were placed post-treatment, occlusal adjustment was performed as previously described (Mizutani *et al.*, 2016), and the patients received instructions and training in daily supragingival plaque control measures and systematic periodontal maintenance care after surgical healing.

Patients receiving ultrasonic root debridement alone were treated for 60 to 120 seconds on microbiologically-sampled tooth sites using the same ultrasonic scaling instrumentation, settings, and irrigation/cooling solution as employed for LANAP -treated patients. Additional time was spent instrumenting adjacent tooth surfaces as needed. These patients were also given instructions and training in daily supragingival plaque control, and subsequently subjected to additional non-surgical and surgical periodontal therapy as needed.

A single experienced periodontist with LANAP surgical protocol training and manufacturer licensure (author TKM) performed all clinical examination and treatment procedures on the patients.

Microbiological Procedures

Using a standardized sampling protocol (Rams *et al.*, 1996; Tabanella *et al.*, 2009), subgingival plaque biofilm samples were obtained from 2 non-adjacent periodontal sites with periodontal probing depths of ≥ 6 mm and bleeding on probing on a single tooth in each patient. Among LANAP-treated patients, microbiologically-sampled teeth included 1 incisor, 4 canines, 14 premolars, and 1 molar (non-furcation sites only), whereas 1 canine, 4 premolars, and 1 molar (non-furcation sites only) were microbiologically-sampled in patients subjected to ultrasonic root debridement alone.

After isolation with cotton rolls, and removal of saliva and supragingival deposits, 2 sterile, absorbent, paper points (Johnson & Johnson, East Windsor, NJ, USA) were advanced to probing depth for approximately 10 seconds into each of the 2 selected periodontal sites in each patient, both before and immediately after completion of either LANAP surgery or ultrasonic root debridement alone. Particular care was used to timely introduce and remove paper points from LANAP-treated subgingival sites prior to complete formation of a fibrin clot at the tooth-gingival flap interface after administration of the second apical-coronal Nd:YAG laser pass, and prior to any soft tissue compression and occlusal adjustment procedures. Upon removal, paper points from the 2 periodontal sites per patient were pooled in a glass vial containing anaerobically prepared and stored VMGA III transport medium (Dahlén *et al.*, 1993). The subgingival samples were transported within 24 hours to a commercial oral microbiology laboratory (Oral Microbiology Testing Laboratory, University of Southern California School of Dentistry, Los Angeles, CA, USA), which was licensed for high-complexity bacteriological analysis by the California Department of Public Health. All microbiology laboratory procedures were paid for on a fee-for-service basis and performed by personnel that were blinded to the clinical status of the patients, the nature of their periodontal treatment, and their inclusion in the present retrospective analysis.

At the laboratory, the subgingival specimens were processed as previously described (Rams *et al.*, 1996; Tabanella *et al.*, 2009), with dilution aliquots of the samples spread onto nonselective enriched Brucella blood agar primary isolation plates incubated at 35°C for 10 days in an anaerobic chamber (Coy Laboratory Products, Ann Arbor, MI, USA) containing 85% N_2-10% H_2-5% CO_2, and onto selective trypticase soy-bacitracin-vancomycin medium with incubation at 35°C for 4 days in ambient air plus 10% CO_2. Organisms examined for in the microbiological testing were *A. actinomycetemcomitans*,

P. intermedia/nigrescens, *P. micra*, *Porphyromonas gingivalis*, *Dialister pneumosintes*, *Tannerella forsythia*, *Campylobacter* species, *Fusobacterium* species, enteric gram-negative rods/pseudomonads, and *Candida* species, which were identified using presumptive phenotypic criteria previously described (Rams *et al.*, 1996; Tabanella *et al.*, 2009). The percentage recovery of each bacterial species per patient was calculated using colony counts of each organism in relation to total subgingival anaerobic viable counts, as determined from nonselective blood agar primary isolation plates.

Data Analysis

Descriptive analyses were used to calculate mean patient age and probing depths of microbiologically-sampled periodontal sites, SD values, and the presence and proportional cultivable recovery of bacterial species among patients. The evaluated periodontal pathogens were grouped for reporting purposes into subgingival clusters (red complex, orange complex, and other species), as previously described (Socransky *et al.*, 1998). Cumulative subgingival proportions (Carvalho *et al.*, 2005; Page and Rams, 2013) for cultivable red and orange complex species were determined by summing species data for each patient and calculating the total mean value across all patients.

McNemar's non-parametric test with Yates' continuity correction assessed the immediate effects of LANAP surgery or ultrasonic root debridement alone on subgingival presence of the evaluated periodontal pathogens. The nonparametric Wilcoxon matched pairs signed-rank test evaluated changes in mean cumulative subgingival proportions of cultivable red and orange complex species per patient. A p - value of ≤ 0.05 was required for statistical significance. Data analysis was performed using a 64-bit statistical software package (STATA/SE 14.2 for Windows, StataCorp PL, College Station, TX, USA).

Results

Mean probing depths of microbiologically-sampled tooth surfaces were 7.4 ± 1.1 (SD) mm (range 6 to 11 mm) in LANAP-treated patients, and 7.7 ± 1.5 (SD) mm (range 6 to 10 mm) in patients treated with ultrasonic root debridement alone. No adverse clinical events or side effects were noted by the treating periodontist, or reported by the patients, with either treatment procedure.

Table 1 presents the cultivable recovery of bacterial species before and immediately after completion of LANAP surgery. Subgingival presence of *P. gingivalis* and *T. forsythia* was significantly decreased by LANAP surgery ($p \leq 0.05$, McNemar's test), with 90% of *P. gingivalis* and 82.4% of *T. forsythia* culture-positive patients becoming culture-negative for the species immediately post-LANAP surgery. Subgingival presence of all evaluated orange complex species was also significantly reduced by LANAP surgery ($p \leq 0.05$, McNemar's test). A total of 17 (85%) of the 20 patients treated with LANAP surgery were culture-negative for all evaluated periodontal pathogens in immediate post-treatment subgingival samples. Among all LANAP-treated patients, mean patient cumulative subgingival proportions of cultivable red and orange complex species were significantly decreased from 16.5 ± 5.4 (SD) % at pre-treatment to 2.3 ± 5.7 (SD) % immediately after LANAP surgery ($p = 0.0001$, Wilcoxon signed-rank test). However, in 3 (15%) patients, immediate post-treatment samples after LANAP surgery remained culture-positive with most of the same periodontal bacterial pathogens and similar cultivable subgingival proportions as detected at baseline.

Table 2 presents the cultivable recovery of bacterial species before and immediately after ultrasonic root debridement alone therapy. No statistically significant differences were found for any evaluated species in their subgingival presence between baseline and immediate post-treatment samples after the completion of ultrasonic root debridement alone (all p - values > 0.05, McNemar's test). Two patients initially positive for subgingival *P. gingivalis* became culture-negative for the species after ultrasonic root debridement alone. Only 1 (16.7%) of 6 patients subjected to ultrasonic root debridement alone were culture-negative for red and orange complex species in immediate post-treatment subgingival samples. The other 5 patients remained culture-positive for most subgingival bacterial pathogens detected at baseline. Among patients treated with ultrasonic root debridement alone, mean patient cumulative subgingival proportions of cultivable red and orange complex species were not significantly decreased, with 6.8 ± 2.6 (SD) % recovered at pre-treatment, and 5.4 ± 3.5 (SD) % immediately post-treatment ($p > 0.05$, Wilcoxon signed-rank test).

Discussion

This report provides the first data on the antimicrobial effects of the LANAP surgical protocol on subgingival biofilms in deep human periodontal pockets. Periodontal pathogens belonging to the red and orange complexes, which are strongly associated with severe progressive periodontitis (Socransky *et al.*, 1998; Rams and van Winkelhoff, 2017), were significantly reduced in their subgingival presence and cultivable proportions immediately after completion of LANAP surgery, with 85% of LANAP-treated patients culture-negative for evaluated periodontal pathogens in immediate post-treatment subgingival biofilm samples. These pronounced changes in subgingival microbial community structure may contribute to the favorable clinical and radiographic treatment outcomes reported by others performing LANAP surgery (Harris *et al.*, 2004; Harris *et al.*, 2014; Yukna *et al.*, 2007; Tilt, 2012; Nevins *et al.*, 2012;

Table 1. Number of patients testing culture-positive, and mean proportional cultivable recovery (SD), of subgingival species in culture-positive patients, before and immediately after LANAP surgery on deep periodontal pockets in 20 adults with periodontitis.

Subgingival species		Pre-treatment	Immediately post-LANAP surgery
Red cluster complex species:			
Porphyromonas gingivalis	n	9	[1]*
	%	3.4 (0.6)	2.7
Tannerella forsythia	n	16	2 [1]*
	%	3.7 (1.1)	3.5 (0.4)
Orange cluster complex species:			
Campylobacter species	n	18	2*
	%	3.4 (0.8)	4.2 (0.5)
Eubacterium species	n	6	0*
	%	2.7 (0.7)	0
Fusobacterium species	n	18	3*
	%	5.3 (1.1)	5.7 (0.4)
Parvimonas micra	n	10	[1]*
	%	3.7 (1.4)	3.1
Prevotella intermedia/nigrescens	n	8	1*
	%	4.4 (1.0)	4.6
Other species:			
Beta-hemolytic streptococci	n	0	0
	%	0	0
Dialister pneumosintes	n	5	1 [1]
	%	3.7 (0.6)	3.4 (1.6)
Eikenella corrodens	n	4	0
	%	5.5 (1.8)	0
Gram-negative enteric rods/pseudomonads	n	4	0
	%	4.5 (0.7)	0

* = Statistically significant change from pre-treatment, $p \leq 0.05$, McNemar test
(n) = number of patients culture-negative for species at pre-treatment, but culture-positive immediately post-treatment

Nevins *et al.* 2014; Brown, 2013), and are consistent with enhanced clinical treatment results noted when red and orange complex periodontal pathogens are markedly suppressed or eradicated during the performance of other types of periodontal regeneration surgery (Machtei *et al.*, 1994; Nowzari *et al.*, 1995; Heitz-Mayfield *et al.*, 2006).

In contrast, treatment with ultrasonic root debridement alone failed to induce statistically significant differences between baseline and immediate post-treatment subgingival biofilm samples for any of the evaluated periodontal pathogens. These findings are in agreement with scanning electron microscopic observations of intact mini-colonies of bacterial biofilms persisting on teeth with deep periodontal pockets immediately after non-surgical ultrasonic root debridement (Breininger *et al.*, 1987). Similarly, manual root scaling in deep periodontal pockets also failed to induce statistically significant reductions in the subgingival presence of cultivable periodontal pathogens immediately post-treatment (Rhemrev *et al.*, 2006). Another study found all red and orange complex species to still be DNA probe-positive, but numerically lower, in post-treatment subgingival specimens removed immediately after ultrasonic and manual curette instrumentation on periodontitis patients (Uzel *et al.*, 2011). In contrast, better immediate post-treatment reductions in subgingival microorganisms may occur in more moderate periodontal pockets. Slot *et al.* (2011) reported an approximately two-thirds immediate reduction in the number of cultivable red and orange complex species strains in moderate periodontal pockets (mean 5.2 mm probing depth) after completion of combined ultrasonic and manual root instrumentation. Wennström *et al.* (2011) found about a one-half immediate reduction in detection of orange complex species strains with checkerboard DNA-DNA hybridization after 30 seconds of ultrasonic instrumentation of residual moderate periodontal pockets (mean 5.7 mm probing depth) within treated periodontitis patients undergoing supportive periodontal recall care. Overall, the present report, along with most previous studies (Breininger *et al.*, 1987; Rhemrev *et al.*, 2006; Uzel *et al.*, 2011), support the conclusion by Rhemrev *et al.* (2006) that "subgingival mechanical cleaning in itself has a limited effect in actually removing bacteria", particularly in regard to deep periodontal pockets.

Table 2. Number of patients testing culture-positive, and mean proportional cultivable recovery (SD), of subgingival species in culture-positive patients, before and immediately after ultrasonic root debridement alone on deep periodontal pockets in 6 adults with periodontitis.

Subgingival species		**Pre-treatment**	**Immediately post- ultrasonic root debridement**
Red cluster complex species:			
Porphyromonas gingivalis	*n*	2	0
	%	0.8 (0.4)	0
Tannerella forsythia	*n*	1	[2]
	%	1.5	1.0 (0.1)
Orange cluster complex species:			
Campylobacter species	*n*	5	4
	%	2.6 (0.6)	2.6 (1.7)
Eubacterium species	*n*	0	[1]
	%	0	1.3
Fusobacterium species	*n*	6	4
	%	2.7 (1.3)	3.5 (0.6)
Parvimonas micra	*n*	5	3
	%	1.7 (0.6)	1.4 (0.2)
Prevotella intermedia/nigrescens	*n*	1	[1]
	%	0.5	0.5
Other species:			
Beta-hemolytic streptococci	n	3	3 [1]
	%	2.5 (1.0)	3.5 (1.2)
Dialister pneumosintes	*n*	1	1
	%	3.2	2.1
Eikenella corrodens	*n*	3	1
	%	1.6 (0.6)	1.3
Gram-negative enteric rods/pseudomonads	*n*	3	2
	%	1.7 (1.2)	3.2 (2.6)

* = Statistically significant change from pre-treatment, $p \leq 0.05$, McNemar test
(n) = number of patients testing culture-negative for species at pre-treatment, but culture-positive immediately post-treatment

The present analysis did not determine why an enhanced antimicrobial effect on periodontal pathogens was observed immediately after LANAP surgery in comparison to ultrasonic root debridement alone. Nd:YAG lasers used at various operational settings may exert bactericidal activity against many periodontal pathogens as a result of thermal heating and/or bacterial chromophore absorption of laser light (Cobb *et al.*, 1992; Neill and Mellonig, 1997; Harris and Yessik, 2004; Kranendonk *et al.*, 2010; Gokhale *et al.*, 2010; Pirnat *et al.*, 2011; Giannelli *et al.*, 2012; Vescovi *et al.*, 2013; Harris *et al.*, 2016; Harris and Reinisch, 2016). It is not known if the Nd:YAG laser, at settings used in the present patient case series, is alone capable of suppressing or eradicating mixed bacterial populations in mature subgingival biofilms. Ting *et al.* (2014) reported, immediately after Nd:YAG laser treatment alone at lower energy settings than employed in the present patient case series, a complete absence of microorganisms within periodontal pockets and adjacent connective tissues in 100% of 14 human tissue biopsies examined with scanning electron microscopy. In addition to bactericidal effects of Nd:YAG laser light, the gingival flap reflection created by the first Nd:YAG laser pass in LANAP surgery also enhances access to subgingival biofilms and increases the efficacy of mechanical root instrumentation. Additional evaluations are needed to better ascertain the relative contributions of laser light and mechanical root instrumentation in altering subgingival biofilms when used in LANAP surgery.

The presented patient case series has several limitations. The data was obtained without treatment group randomization, rather than through a randomized clinical trial to better minimize potential study bias. No microbiological or clinical assessments were systematically made beyond the immediate completion of the two periodontal treatments. As a result, it was not possible to determine if LANAP surgery provided

better microbiological or clinical outcomes than conventional mechanical root debridement over a longer post-treatment period. In contrast to the application of Nd:YAG lasers in LANAP surgery, no significant additional benefits have been reported to date when Nd:YAG lasers are used as an adjunct to conventional non-surgical periodontal therapy (Slot et al., 2011; Smiley *et al.*, 2015; Cobb, 2017). Additionally, changes in total and species-specific subgingival anaerobic viable counts were not provided by the microbiology laboratory and remain unreported. Cultivation, instead of more sensitive molecular microbiological methods (Rams and van Winkelhoff, 2017), was used to assess treatment-induced changes in subgingival biofilms. Post-treatment subgingival biofilm specimens may have been adversely altered by paper points used in sampling becoming saturated with blood as they were advanced apically into treated periodontal pockets (Angelov *et al.*, 2009). However, 3 (15%) patients after completion of LANAP surgery, and 5 (83.3%) patients treated with ultrasonic root debridement alone, remained culture-positive with most of the same periodontal pathogens and similar cultivable subgingival proportions as detected at baseline, suggesting that this potential effect, if present, did not uniformly occur. Why these few deep periodontal pockets remained culture-positive for periodontal pathogens upon completion of LANAP surgery was not determined. No ultrastructural assessments were made to detect potential laser-induced alterations to cementum or dentin on treated tooth root surfaces (Cobb *et al.*, 1992), even though no root surface damage resulting from LANAP surgery was previously reported by Yukna *et al.* (2007). No comparisons were made to other types of dental lasers, or to other types of periodontal surgery. Appropriately designed research studies on the LANAP surgical protocol are needed to address these issues.

Conclusions

In conclusion, this patient case series showed that the LANAP surgical treatment protocol, but not conventional ultrasonic root debridement, immediately suppressed red and orange complex periodontal pathogens below culture detection limits in most deep human periodontal pockets.

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LANAP is patented and a registered trademark of Millennium Dental Technologies, Inc., Cerritos, CA. Millennium Dental Technologies, Inc., Cerritos, CA, had no role, either directly or indirectly, in the conceptualization or inception of this patient case series, funding of it, accrual or analysis of the data, preparation or approval of this manuscript, or the decision to publish the results of the data analysis. The evaluations and conclusions made in this report are solely those of the authors.

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