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Immediate effect of Nd:YAG laser monotherapy on subgingival periodontal pathogens: a pilot clinical study

Thomas K. McCawley [1], **Mark N. McCawley** [1], **Thomas E. Rams** [2,3*]

[1]McCawley Center for Laser Periodontics and Implants, Ft. Lauderdale, FL, USA
[2]Department of Periodontology and Oral Implantology, Temple University School of Dentistry, Philadelphia, PA, USA
[3]Department of Microbiology and Immunology, Temple University School of Medicine, Philadelphia, PA, USA

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***Correspondence:**
Thomas E. Rams
Department of Periodontology and Oral Implantology, Temple University School of Dentistry, 3223 North Broad Street, Philadelphia, PA 19140, USA.
Email: trams@temple.edu
Tel: +1-215-707-2941
Fax: +1-215-707-4223

ORCID iDs
Thomas K. McCawley
https://orcid.org/0000-0002-1539-5530
Mark N. McCawley
https://orcid.org/0000-0001-6072-7189
Thomas E. Rams
https://orcid.org/0000-0002-5735-717X

Author Contributions
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ABSTRACT

Purpose: This pilot study assessed the immediate *in vivo* effect of high peak pulse power neodymium-doped yttrium aluminum garnet (Nd:YAG) laser monotherapy on selected red/orange complex periodontal pathogens in deep human periodontal pockets.
Methods: Twelve adults with severe periodontitis were treated with the Laser-Assisted New Attachment Procedure (LANAP®) surgical protocol, wherein a free-running, digitally pulsed, Nd:YAG dental laser was used as the initial therapeutic step before mechanical root debridement. Using a flexible optical fiber in a handpiece, Nd:YAG laser energy, at a density of 196 J/cm^2 and a high peak pulse power of 1,333 W/pulse, was directed parallel to untreated tooth root surfaces in sequential coronal-apical passes to clinical periodontal probing depths, for a total applied energy dose of approximately 8–12 joules per millimeter of periodontal probing depth at each periodontal site. Subgingival biofilm specimens were collected from each patient before and immediately after Nd:YAG laser monotherapy from periodontal pockets exhibiting ≥6 mm probing depths and bleeding on probing. Selected red/orange complex periodontal pathogens (*Porphyromonas gingivalis*, *Tannerella forsythia*, *Prevotella intermedia/nigrescens*, *Fusobacterium nucleatum*, *Parvimonas micra*, and *Campylobacter* species) were quantified in the subgingival samples using established anaerobic culture techniques.
Results: All immediate post-treatment subgingival biofilm specimens continued to yield microbial growth after Nd:YAG laser monotherapy. The mean levels of total cultivable red/orange complex periodontal pathogens per patient significantly decreased from 12.0% pre-treatment to 4.9% (a 59.2% decrease) immediately after Nd:YAG laser monotherapy, with 3 (25%) patients rendered culture-negative for all evaluated red/orange complex periodontal pathogens.
Conclusions: High peak pulse power Nd:YAG laser monotherapy, used as the initial step in the LANAP® surgical protocol on mature subgingival biofilms, immediately induced significant reductions of nearly 60% in the mean total cultivable red/orange complex periodontal pathogen proportions per patient prior to mechanical root instrumentation and the rest of the LANAP® surgical protocol.

Keywords: Laser therapy; Microbiology; Periodontal diseases; Periodontal pocket; Periodontitis; Surgery

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Conflict of Interest

The authors declare no potential conflict of interest relevant to this article. Dr. Thomas K. McCawley received reimbursement for expenses related to his participation in 2010–2012 as a co-investigator on a clinical trial funded by Millennium Dental Technologies, Inc., which evaluated the LANAP® surgical protocol and other forms of surgical and non-surgical periodontal therapy. Dr. Rams is certified as a LANAP® surgical protocol instructor by the Institute for Advanced Laser Dentistry, Cerritos, CA, USA, a non-profit educational/research entity affiliated with Millennium Dental Technologies, Inc. Neither Millennium Dental Technologies, Inc. nor the Institute for Advanced Laser Dentistry, had any role in the conceptualization or design of the present study; in collection, analyses, or interpretation of data; in writing of the manuscript, or in the decision to publish the results.

INTRODUCTION

The Laser-Assisted New Attachment Procedure (LANAP®) is an emerging, but controversial, minimally-invasive periodontal disease surgery protocol that improves the clinical and radiographic status of human periodontitis lesions [1-5]. Histologically, the LANAP® surgical protocol promotes periodontal regeneration on apical portions of severely diseased tooth root surfaces, where a new functionally oriented periodontal attachment apparatus may form, composed of new cementum and alveolar bone with inserting periodontal ligament fibers [1,2]. In 2016, the United States Food and Drug Administration, after reviewing manufacturer safety and effectiveness data, cleared for marketing purposes a laser manufacturer claim (K151763) of true periodontal regeneration in human periodontitis lesions as an indication for the LANAP® surgical protocol [6]. However, long-term comparative outcomes of LANAP® surgery with other forms of surgical and non-surgical periodontal therapy in the treatment of periodontitis are not presently available in the dental scientific literature.

The use of a free-running, digitally pulsed, neodymium-doped yttrium aluminum garnet (Nd:YAG) solid-state crystal dental laser is an essential component of the LANAP® surgical protocol [5]. In the protocol's first step, Nd:YAG laser passes are advanced into periodontal pockets, prior to any other therapeutic procedures, with short pulse durations and a high peak pulse power setting, which is defined as the maximum optical energy level reached during a single laser pulse [7]. In this step, the aim of Nd:YAG laser treatment is to selectively photoablate inflamed pocket epithelium ("de-epithelialization") [8,9]; initiate reflection of a gingival surgical access flap similar to that created by an excisional new attachment surgical procedure [5]; establish a dry and bloodless field [10], reduce dental calculus adherence to tooth root surfaces, facilitating its removal [10]; and potentially kill bacteria in subgingival biofilms [5]. Additional Nd:YAG laser passes are applied a second time later in the LANAP® surgical protocol, but with longer pulse durations and a lower peak pulse power level, and only after mechanical root debridement and osseous modification/decortication is completed, in order to thermally induce (congeal) a fibrin blood clot at the tooth-gingival flap interface for hemostasis and post-treatment soft tissue stabilization without sutures [5,11]. Occlusal adjustments are also performed in the LANAP® surgical protocol after completing Nd:YAG laser irradiation to eliminate heavy, excursive, or depressive tooth contacts and fremitus to help maintain post-treatment wound stability and encourage passive tooth eruption [5].

In a previous clinical study, red/orange complex periodontal bacterial pathogens, which are strongly associated with severe forms of human periodontitis [12], showed significantly reduced subgingival prevalence and cultivable proportional levels immediately after completion of the entire LANAP® surgical protocol [13]. Subgingival biofilms evaluated immediately post-treatment revealed that 85% of LANAP®-treated severe periodontitis patients were culture-negative for red/orange complex periodontal pathogens, whereas no statistically significant changes in the organisms were found among patients treated with conventional ultrasonic tooth root debridement alone [13].

However, the extent to which high peak pulse power Nd:YAG laser irradiation alone, as the first step in the LANAP® surgical protocol, alters pathogenic subgingival biofilms is unclear [13]. It is not known how well this laser energy as monotherapy, prior to mechanical root debridement, is capable of immediately suppressing or eradicating putative periodontal bacterial pathogens in mature subgingival biofilms. To address this unresolved question,

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the purpose of this pilot clinical study was to assess the immediate *in vivo* effect of high peak pulse power Nd:YAG® laser monotherapy on the cultivable presence and levels of selected red/orange complex periodontal pathogens in deep human periodontal pockets.

MATERIALS AND METHODS

Patients

This study involved a retrospective analysis of de-identified patient records at a private periodontal practice in Ft. Lauderdale, FL, USA. The Temple University Human Subjects Institutional Review Board reviewed this study and determined that no action was applicable on their part, since no investigator-study patient contact, interaction, or intervention was involved beyond a retrospective secondary analysis of archived dental practice data not linked to the identification of any person(s).

Twelve consecutive adult patients were identified (5 men and 7 women; mean±standard deviation [SD] age 52.6±16.3 years; age range, 26–72 years) who had been diagnosed with localized to generalized severe chronic periodontitis (equivalent to localized to generalized stage III/grade B periodontitis) [14] and treated with the LANAP® surgical protocol. For each of these patients, subgingival biofilm specimens for microbiological testing were collected prior to treatment by the treating periodontist as part of his normal diagnostic procedures, and again after initial Nd:YAG laser therapy in an attempt to help identify potential antimicrobial agent needs for the patients. This pre-existing microbiological data thus provided the opportunity for a secondary assessment of the immediate *in vivo* effect of the initial Nd:YAG laser therapy on selected subgingival microorganisms.

The identified patients were in good systemic health, had at least 22 teeth, and had not received any systemic antibiotic therapy within 3 months prior to LANAP® surgery. Persons with a history of diabetes mellitus, 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 inclusion in the study, as were individuals with localized molar-incisor forms of periodontitis, gingival pain, or acute periodontal conditions. Each of the patients had provided written informed consent, in compliance with the Helsinki Declaration of 1975, as revised in 2000, for their periodontal therapy. A single periodontist with LANAP® surgical protocol training, manufacturer licensure, and >25 years of Nd:YAG dental laser-related periodontal patient care experience (author T.K.M.) performed all diagnostic assessments, microbiological sampling, and treatment procedures on the patients.

Nd:YAG laser monotherapy

As the first therapeutic step in the LANAP® surgical protocol, a free-running, digitally pulsed, 1,064-nm wavelength, Nd:YAG dental laser (Periolase MVP-7, Millennium Dental Technologies, Inc., Cerritos, CA, USA), with an attached 360-μm diameter round, flexible, optical glass fiber held in a metal handpiece (TrueFlex® handpiece and cannula, Millennium Dental Technologies, Inc.), was used to apply high peak pulse power Nd:YAG laser monotherapy to periodontal sites in the patients. The laser device was set at 4.0 W average power, a 20-Hz pulse frequency rate, and a 150-μs pulse duration, which provided a power density (irradiance) of 3,930 W/cm^2, an energy density (fluence) at the end of the optical fiber of 196 J/cm^2, and a peak pulse power of 1,333 W/pulse. Per manufacturer instructions, the

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optical glass fiber tip was cleaved prior to each clinical use. A power meter and joule counter built into the Periolase MVP-7 laser unit were used to verify the optical fiber tip power output to within 0.1 W of the designated instrument setting and to tabulate laser light doses applied to periodontal pockets, respectively. Laser wavelength-specific protective eyewear was worn by dental personnel and patients during laser use. The optical fiber tip was advanced, prior to any mechanical root debridement, parallel to untreated tooth root surfaces in sequential coronal-apical laser passes to the extent of clinical periodontal probing depths, delivering a total energy dose of approximately 8–12 joules per millimeter of periodontal probing depth to each treated periodontal site [15]. The total laser irradiation application time at each periodontal site with the above Nd:YAG laser settings varied depending upon its periodontal probing depth, and is estimated to have been approximately 2-3 seconds per millimeter of periodontal probing depth. Following microbiological re-sampling of subgingival biofilms immediately after completion of the Nd:YAG laser monotherapy, the remaining portions of the LANAP® surgical protocol were carried out on the patients as previously described [5,13].

Subgingival biofilm sampling and culture analysis

Two non-adjacent periodontal pockets with ≥6-mm probing depths and bleeding on probing at a single tooth in each patient were microbiologically sampled. The teeth sampled included 1 maxillary incisor, 1 maxillary canine, 9 maxillary premolars, and 1 mandibular molar (non-furcation tooth surfaces only). Both before and immediately after completion of Nd:YAG laser monotherapy, 2 sterile, absorbent paper points (Johnson & Johnson, East Windsor, NJ, USA) were advanced for approximately 10 seconds into each of the selected periodontal pockets after isolation with cotton rolls and removal of saliva and supragingival deposits. Care was taken to introduce the paper points into the same periodontal pocket locations per patient for subgingival sampling before treatment and after laser monotherapy. Upon removal from the periodontal sites, the pre-treatment and post-laser monotherapy paper points from each patient were pooled separately into different glass vials containing anaerobically prepared and stored VMGA III transport medium [16], resulting in 1 pre-treatment and 1 post-laser monotherapy microbial sample per patient. The subgingival samples were transported within 24 hours, and processed on a fee-for-service basis, to the Oral Microbiology Testing Laboratory, University of Southern California School of Dentistry, Los Angeles, CA, USA (for 7 pairs of patient samples), and the Oral Microbiology Testing Service Laboratory, Temple University School of Dentistry, Philadelphia, PA, USA (for 5 pairs of patient samples), which were licensed for high-complexity bacteriological analysis by the California Department of Public Health, and the Pennsylvania Department of Health, respectively. The microbiology laboratories processed the subgingival specimens using the same microbial culture protocols [17,18], and shared a high level of joint agreement in their isolation and identification of red/orange complex species in subgingival biofilm samples [19]. In brief, sample dilution aliquots were spread onto nonselective enriched *Brucella* blood agar plates and incubated anaerobically at 37°C for 10 days. The microbial species identified in this study, with methods previously described [17,18,20,21], were *Porphyromonas gingivalis* and *Tannerella forsythia* among red complex species, and *Prevotella intermedia/nigrescens*, *Parvimonas micra*, *Fusobacterium nucleatum*, and *Campylobacter* species among orange complex species. The percentage recovery of each bacterial species per patient was calculated using the colony count of each organism in relation to the total subgingival anaerobic viable count as determined from the nonselective enriched *Brucella* blood agar plates. All microbiology laboratory procedures were performed independently by personnel who were blinded to patients' clinical status, the nature of their periodontal treatment, and their inclusion in the present retrospective data analysis.

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In a separate analysis to evaluate the potential effects of subgingival sampling alone on microbiological findings, duplicate paper point subgingival biofilm samples were removed from deep periodontal pockets within 30 minutes of each other without any intervening intervention from 3 adults with severe periodontitis who did not undergo Nd:YAG laser treatment, and processed as described above by the Oral Microbiology Testing Service Laboratory at Temple University School of Dentistry.

Data analysis

Descriptive analyses were used to calculate the mean patient age and clinical probing depths of microbiologically-sampled periodontal sites, SD values, and the presence and proportional cultivable recovery of red/orange complex species per patient that demonstrated moderate to heavy microbial growth (≥1% of the total cultivable count) [13] in pre-treatment subgingival biofilm samples. The total mean cultivable subgingival proportions of red/orange complex species per patient in the pre-treatment and post-laser monotherapy microbial samples were determined by summing together individual species data for each patient, and then calculating total mean values across all patients, as previously described [13].

The McNemar non-parametric test with the Yates continuity correction was used to assess the immediate effects of Nd:YAG laser monotherapy on the subgingival presence or absence of test species. The nonparametric Wilcoxon matched-pairs signed-rank test was used to evaluate immediate post-treatment changes from pre-treatment values in the mean total cultivable subgingival proportions of red/orange complex species per patient, as well as for individual bacterial species. A Pvalue≤0.05 indicated statistical significance. Data analysis was performed using a 64-bit statistical software package (STATA/SE 16.0 for Windows, StataCorp PL, College Station, TX, USA).

RESULTS

The mean periodontal probing depth of the microbiologically-sampled tooth surfaces was 7.0±1.0 (SD) mm (range 6–9 mm) in the study patients. No adverse clinical events or side effects were noted by the treating periodontist, or reported by the patients, as a result of the subgingival high peak pulse power Nd:YAG laser monotherapy.

All subgingival biofilm specimens yielded microbial growth before and after laser monotherapy. Mean total anaerobic viable counts averaged $1.8\times10^{8}\pm3.4\times10^{7}$ (SD) organisms/ml of subgingival sample prior to treatment, and decreased to $1.0\times10^{8}\pm7.8\times10^{7}$ (SD) immediately after Nd:YAG laser treatment, but were not significantly different from pre-treatment levels (P>0.05, Wilcoxon signed-rank test).

Table 1 presents the subgingival presence of red/orange complex species in patients before and immediately after Nd:YAG laser monotherapy. Three (25%) patients were rendered culture-negative for all evaluated red/orange complex periodontal pathogens in immediate post-treatment subgingival samples after Nd:YAG laser monotherapy. All evaluated individual red/orange complex species showed a reduced subgingival presence immediately after Nd:YAG laser monotherapy, but the decreases for each individual species were not statistically significant compared to the pre-treatment numbers (all Pvalues>0.05, McNemar test).

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Table 1. Presence of red/orange complex species before and immediately after neodymium-doped yttrium aluminum garnet laser monotherapy

Subgingival species	Present pre-treatment	Present immediately post-treatment
Red complex species		
Porphyromonas gingivalis	2	1
Tannerella forsythia	3	2
Orange complex species		
Campylobacter species	3	2
Fusobacterium nucleatum	11	9
Parvimonas micra	9	6
Prevotella intermedia/nigrescens	2	1
Total red/orange complex species per patient[a]	12	9

Values represent the number of culture-positive patients for a given species.
[a]Represents the per-patient presence of 1 or more of the evaluated red/orange complex species.

Table 2. Subgingival percentage levels of red/orange complex species before and immediately after neodymium-doped yttrium aluminum garnet laser monotherapy

Subgingival species	Cultivable percentages per patient[a]	
	Percent pre-treatment[b]	Percent immediately post-laser treatment[c]
Red complex species		
Porphyromonas gingivalis	4.9±2.4	4.4±6.2
Tannerella forsythia	5.8±7.8	1.5±1.7
Orange complex species		
Campylobacter species	2.4±0.3	1.5±1.3
Fusobacterium nucleatum	4.9±6.0	2.1±1.5
Parvimonas micra	4.8±4.1	1.8±1.7
Prevotella intermedia/nigrescens	5.4±6.2	1.0±1.4
Total red/orange complex species per patient	12.0±9.6	4.9±5.4[d]

Values are presented as mean±standard deviation.
[a]Calculated as the average percentage of colony-forming units recovered for each species among total cultivable anaerobic viable counts; [b]Calculated for species-positive patients; [c]Calculated for patients positive for a species at pre-treatment; [d]Value significantly different from pre-treatment, P=0.002.

Table 2 shows the average subgingival percentage levels of red/orange complex species in patients before and immediately after Nd:YAG laser monotherapy. The subgingival cultivable percentages of all individual red/orange complex periodontal pathogens decreased immediately after laser monotherapy, but the decreases for each individual species were not statistically significant compared to the pre-treatment values (all P-values>0.05, Wilcoxon signed-rank test). In contrast, when all evaluated red/orange complex species were considered collectively within each study patient, the mean total levels of red/orange complex species per patient averaged 12.0% per patient at pre-treatment, and significantly decreased to 4.9% per patient immediately after Nd:YAG laser monotherapy (a 59.2% decrease from pre-treatment) (P=0.002, Wilcoxon signed-rank test).

The duplicate subgingival specimens removed from deep periodontal pockets in 3 severe periodontitis patients without any intervening intervention revealed the same prevalence and similar subgingival levels (16.9% vs. 18.4%) of red/orange complex species.

DISCUSSION

Nd:YAG lasers have been suggested for decontamination of infected human periodontal pockets [5], since *in vitro* they exert marked bactericidal activity against many red/orange complex periodontal pathogens, including *P. gingivalis*, *T. forsythia*, *P. intermedia/nigrescens*, *P. micra*, and *F. nucleatum* [22-25]. The present *in vivo* study findings are consistent with these *in vitro* antimicrobial effects, as the high peak pulse power Nd:YAG laser monotherapy (1,333

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W/pulse) significantly decreased the mean total cultivable levels of red/orange complex periodontal pathogens per patient from 12.0% pre-treatment to 4.9% immediately post-treatment (a 59.2% decrease), with 3 (25%) patients rendered culture-negative for all evaluated red/orange complex species. These microbial culture findings are also consistent with scanning electron microscopic observations of gingival tissue biopsies, where epithelial and gingival connective tissue-associated bacterial morphotypes were no longer present immediately after Nd:YAG laser monotherapy [9]. Microbial culture was used in this study to recover viable subgingival bacteria surviving immediately after Nd:YAG laser light emission, instead of more sensitive molecular DNA hybridization and amplification methods, such as polymerase chain reaction and next-generation sequencing, which are able to detect smaller numbers and a wider range of microbial species than culture, including uncultivated and/or unrecognized microbial phylotypes, but are unable to differentiate between living and dead microorganisms [26]. The present study is the first to evaluate *in vivo* the immediate post-treatment bactericidal effects of high peak pulse power Nd:YAG laser monotherapy within periodontal pockets without potential confounding by the antimicrobial impact of concomitant mechanical root debridement or medication treatment procedures.

Several factors may account for the finding that most study patients (75%) remained culture-positive for red/orange complex species, but at lower levels than baseline, immediately after Nd:YAG laser monotherapy. The applied laser light energy dose of approximately 8–12 joules per millimeter of periodontal probing depth at a peak pulse power of 1,333 W/pulse may have been inadequately bactericidal against microbial biofilm-associated red/orange complex species in periodontitis lesions. Since the Nd:YAG laser was directed parallel to the tooth root surfaces, and not perpendicular, the applied laser energy may not have adequately reached or affected some areas of infected root surfaces. Similarly, the beam of Nd:YAG laser light, applied with a 360-μm-diameter round optical fiber, may have been insufficiently applied in successive, adjacent, and non-overlapping subgingival passes to fully cover and contact the extent of microbial biofilm-laden root surfaces. As a result, residual islands of viable bacterial biofilm may have survived lateral to the Nd:YAG laser beam as it was apically introduced into periodontal pockets, as has been found with other types of dental lasers [27,28]. Moreover, microbial biofilms located within root surface irregularities, flutings, concavities, or cementum lacunae; or growing within dental calculus and/or shielded apical to markedly-raised ledges of dental calculus deposits on subgingival tooth surfaces, may have been outside the range and direction of the applied Nd:YAG laser irradiation.

Other studies have examined the microbiological impact of Nd:YAG lasers on human subgingival sites with various study designs, where different laser energy settings, pulse durations, and contact times were used with and without concurrent mechanical root instrumentation or locally-applied antibiotic agents, or in combination with other types of lasers [13,29-38]. Some studies documented enhanced subgingival reductions of putative periodontal pathogens with Nd:YAG lasers as compared to mechanical root debridement alone [29-33], whereas others failed to show statistically significant adjunctive microbiological effects [34-38], particularly when only low peak pulse power Nd:YAG settings of 480 W/pulse [37] or 667 W/pulse [34] were used (calculated from data supplied in papers). However, Nd:YAG laser light as monotherapy, with the application of 240 J at an unreported peak pulse power per periodontal site, was as effective as ultrasonic scaling alone in decreasing subgingival *P. gingivalis* in deep periodontal pockets over a 12-week post-treatment period [31]. Nd:YAG laser subgingival applications at a high peak pulse power (1,250 W/pulse), and subsequently again at a lower peak pulse power (292 W/pulse), in combination

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with an erbium-doped yttrium aluminum garnet laser for periodontal root debridement, better suppressed total subgingival bacterial counts and red/orange complex periodontal pathogens over 6 months than mechanical root debridement alone [33].

Nd:YAG lasers may provide "selective photoantisepsis" by killing periodontopathic *P. gingivalis* and *P. intermedia* species in periodontal pockets, since the absorption of laser energy, and hence susceptibility to associated laser thermal killing effects, at the Nd:YAG 1,064-nm wavelength is estimated to be 100-fold greater in *P. gingivalis* and *P. intermedia* than is likely in surrounding gingival soft tissues [25]. This differential absorption of Nd:YAG laser light between bacterial pathogens and normal gingival tissue offers the possibility of selective ablation of bacterial biofilms in periodontal pockets while limiting damage to adjacent periodontal tissues [25]. In this regard, Nd:YAG laser light was found *in vivo* to markedly reduce or eradicate red/orange complex periodontal pathogens (*P. gingivalis*, *Treponema denticola*, *P. intermedia*, *F. nucleatum*), as well as *Aggregatibacter actinomycetemcomitans*, located internally within gingival tissue epithelial cells, without causing damage to underlying connective tissue or blood microvessels [39]. The application of a high peak pulse power Nd:YAG laser light with short pulse durations, as was employed in this study, also serves to better confine the spread of potentially damaging laser thermal effects into tissues surrounding laser-targeted areas, and helps limit unwanted collateral tissue damage [25].

The present study data help to address the following question: to what extent does high peak pulse power Nd:YAG laser monotherapy, as the first step in the LANAP® surgical protocol, alter pathogenic subgingival biofilms, as compared to the effects of the entire LANAP® surgical protocol? As shown in the present study, mean levels of total cultivable red/orange complex periodontal pathogens per patient significantly decreased by nearly 60% from pre-treatment values immediately after Nd:YAG laser monotherapy, as compared to an approximately 86% reduction (a 26 percentage points additional reduction) from pre-treatment found immediately after completion of the entire LANAP® surgical protocol, as calculated from data previously reported [13]. Additionally, 25% of patients in this study were culture-negative for all evaluated subgingival red/orange complex species immediately after Nd:YAG laser monotherapy. In comparison, 85% of patients (a 60 percentage points additional increase) were previously reported to be totally culture-negative for the same red/orange complex species after completion of the entire LANAP® surgical protocol [13], with only low post-LANAP® treatment levels (mean 2.3% compared to 4.9% in this study) of total subgingival red/orange complex periodontal pathogens, compatible with a lowered risk of progressive periodontal breakdown [17], detected among all treated patients [13]. These data suggest that the pronounced beneficial decreases in red/orange complex periodontal pathogens found after completion of the entire LANAP® surgical protocol [13] are likely due to a combined antimicrobial impact of high peak pulse power in Nd:YAG laser monotherapy, plus subsequent mechanical root debridement, plus additional Nd:YAG laser applications at lower peak pulse power settings (approximately 308 W/pulse), and not merely from the initial Nd:YAG laser irradiation alone. This emphasizes the need to perform all of the recommended steps in the LANAP® surgical protocol to attain the best therapeutic outcomes. It is also important to differentiate between the surgical application of high peak pulse power Nd:YAG laser energy in the present study, and adjunctive use of Nd:YAG and diode lasers at markedly lower peak pulse power settings for "pocket disinfection" during periodontal non-surgical/maintenance care, where clinical and microbiological outcomes to date have failed to provide meaningful benefits to treated patients [28,36,40].

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The present pilot clinical study is limited by its retrospective study design, the lack of additional microbiological or clinical evaluations beyond the immediate post-laser monotherapy treatment point, the absence of comparisons with other Nd:YAG laser energy doses, and the fact that it did not determine the mechanism of antimicrobial action exerted by Nd:YAG laser irradiation (i.e., thermal effects only or specific laser light-chromophore interactions). The small patient sample size and the low pre-treatment prevalence of several red/orange complex species impaired the study's ability to detect statistically significant changes among individual bacterial species after Nd:YAG laser monotherapy, as compared to the collective evaluation of total red/orange complex species per patient. A larger patient sample size, as well as the inclusion of patients with a greater pre-treatment presence and levels of red/orange periodontal pathogens, would increase the likelihood of finding statistically significant individual bacterial changes in response to Nd:YAG laser exposure. Since the remaining steps of the LANAP® surgical protocol were completed once the second microbiological samples were collected, the longevity of the immediate suppression of red/orange complex periodontal pathogens by high peak pulse power Nd:YAG laser monotherapy could not be determined. No comparisons were made to other types of dental lasers or other modes of periodontal surgery. No morphological evaluations were attempted of Nd:YAG laser-treated teeth since no histologic evidence of LANAP® surgical treatment-associated tooth root surface damage was found in prior studies [1,2]. Additional research addressing these issues is warranted.

In conclusion, high peak pulse power Nd:YAG laser monotherapy used as the initial step in the LANAP® surgical protocol on mature subgingival biofilms immediately induced significant reductions of nearly 60% in mean total cultivable red/orange complex periodontal pathogen proportions per patient in the absence of mechanical root instrumentation and the rest of the LANAP® surgical protocol.

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