

UC Irvine

UC Irvine Previously Published Works

Title

Thermal and microstructural effects of nanosecond pulsed Nd:YAG laser irradiation on tooth root surface

Permalink

<https://escholarship.org/uc/item/36z2x85p>

ISBN

9780819417411

Authors

Wilder-Smith, Petra BB
Arrastia-Jitosho, Anna-Marie A
Grill, G
[et al.](#)

Publication Date

1995-05-01

DOI

10.1117/12.207438

Copyright Information

This work is made available under the terms of a Creative Commons Attribution License, available at <https://creativecommons.org/licenses/by/4.0/>

Peer reviewed

Thermal and microstructural effects of nanosecond pulsed Nd:YAG laser irradiation on tooth root surface

Petra Wilder-Smith, Anna Marie Arrastia, Gary Grill, Lih-Huei Liaw, Michael Berns

Beckman Laser Institute and Medical Clinic, University of California, Irvine 92715

ABSTRACT

Plaque, calculus and altered cementum removal by scaling and root planing is a fundamental procedure in periodontal treatment. However, the residual smear layer contains cytotoxic and inflammatory mediators which adversely affect healing. Chemical smear layer removal is also problematic.

In previous investigations effective smear layer removal was achieved using long pulsed irradiation at 1.06 μ . However, laser irradiation was not adequate as an alternative to scaling and root planing procedures and concurrent temperature rises exceeded thermal thresholds for pulpal and periodontal safety.

It was the aim of this study to determine whether nanosecond pulsed irradiation at 1.06 μ could be used as an alternative or an adjunct to scaling and root planing. 60 freshly extracted teeth were divided as follows: 5 control, 5 root planed only, 25 irradiated only, 25 root planed and irradiated. Irradiation was performed at fluences of 0.5-2.7J/cm², total energy densities of 12-300 J/cm², frequencies of 2-10 Hz using the Medlite (Continuum) laser. Irradiation-induced thermal events were recorded using a thermocouple within the root canal and a thermal camera to monitor surface temperatures. SEM demonstrated effective smear layer removal with minimal microstructural effects. Surface temperatures increased minimally (<3C) at all parameters, intrapulpal temperature rises remained below 4 C at 2 and 5 Hz, F<0.5 J/cm². Without prior scaling and root planing, laser effects did not provide an adequately clean root surface.

KEY WORDS: Nd:YAG laser, periodontal, smear layer removal, thermal effects, tooth root

1. INTRODUCTION

The removal of plaque, calculus and altered cementum by means of scaling and root planing is a fundamental procedure in periodontal treatment (1,2). However, a residual surface smear layer is common on root surfaces of teeth after these procedures (3-7). This smear layer contains cytotoxic and inflammatory mediators which adversely affect healing (8-11). Various adjunct chemical treatments of the root surface, particularly citric acid conditioning and topical applications of tetracycline have been suggested for cleaning and detoxification or demineralization of the root surface in order to encourage new attachment formation (1,4-6,11-15).

After citric acid treatment, the smear layer caused by mechanical instrumentation is usually removed, leaving patent dentinal tubules and varying degrees of dentin matrix exposed (16,17). However, in the SEM these dentin tubules show widened, funnel-shaped orifices (4-6,17). Ryan et al (18) observed adverse pulpal responses and bacterial penetration of exposed dentinal tubules on citric acid-treated teeth in cats.

Significant antimicrobial activity against subgingival flora has been achieved using tetracycline hydrochloride (TTC-HCl). Improved clinical results such as significantly increased attachment gain as compared to scaling and root planing procedures alone have also been reported (19). However, Wikesjo et al (20) observed in vitro removal of smear layer and surface demineralization of dentin by TTC-HCl, although possibly to a lesser degree than that observed using citric acid (21).

Adriaens et al (22) demonstrated bacterial invasion in the radicular cementum and dentin of periodontally-diseased, caries-free teeth. Since this bacterial invasion cannot be eliminated by conventional mechanical periodontal treatment, and since conventional methods commonly produce a smear layer which may adversely affect healing, it seems appropriate to combine mechanical periodontal therapy with other adjunct treatment forms in order to render the treated root surface biologically compatible for reattachment with host periodontal tissues.

Although a number of studies have investigated the effect of laser irradiation on dental hard tissues, there have been relatively few attempts to methodically evaluate the use of lasers for periodontal hard tissue therapy, particularly root surface treatment.

Tani and Kawada (23) reported that laser irradiation with an Nd:YAG and a CO2 laser removed the smear layer from the surface of extracted bovine teeth. Ito et al (24) reported that Nd:YAG laser irradiation can effectively remove root surface smear layer after root planing, uncovering dentinal tubules and exposing collagen fibers without widening the orifices of dentinal tubules. However, they used very high power levels (20W) of non-pulsed radiation over relatively short periods of time (0.3-3s), which poses a variety of thermal and practical difficulties in a clinical situation.

Nd:YAG laser irradiation at fairly high frequencies, either alone, or followed by root planing, did not provide achieve a clean and smooth root surface according to studies on extracted teeth by Morlock et al (25). In a similar in vivo study, Cobb et al (26) observed ineffective and patchy removal of deposits on the root surface, associated with areas of cratering and meltdown. A post-treatment decrease in microbial levels was also reported.

The purpose of this study was to determine at which clinically relevant parameters Nd:YAG laser radiation (wavelength = 1064 nm) can be used, either alone, or in conjunction with root planing, to achieve a clean and smooth root surface. Results were assessed using SEM, intra-canal and surface thermography.

2. MATERIALS AND METHODS

2.1 Preparation of Specimens

Sixty freshly-extracted single-rooted, clinically and radiographically healthy human teeth obtained from different patients were cleaned with a soft brush under running water. They were set in individual acrylic blocks with the crown portion of the tooth invested. These samples were then randomly assigned to one of four groups.

Group A: 25 teeth were root planed in an apical-coronal direction using a freshly sharpened curette, applying 35 firm strokes per surface. After root planing, these teeth received laser irradiation.

Group B: 5 teeth were root planed only as described above.

Group C: 25 teeth received laser irradiation only.

Group D: 5 teeth served as control specimens and received no treatment.

All root planing was performed by one operator.

2.2 Laser Treatment

A Q-Switched nano-second pulsed Nd:YAG laser emitting at 1064 nm (Medlite, Continuum Biomedical) was used. Irradiation was performed at fluences of 0.5-2.7 J/cm², total energy densities of 12-300 J/cm², frequencies of 2-10 Hz and a spot size of 3mm. Light was delivered via an articulated arm system. During irradiation the laser handpiece and the tooth were clamped into position. The specific Nd:YAG laser parameters tested are shown in Table 1.

Table 1: Specific Nd: YAG Laser Parameters Tested

Fluence J/cm ²	Hz	Exposure Time (s)	Energy Density (J/cm ²)
0.4	2	15	12
0.4	5	30	60
0.4	5	60	120
0.4	5	120	240
0.5	10	60	300
1.0	5	15	75
2.7	2	30	162

Intra-canal thermal events were monitored using a thermocouple (Philip Type K, 63% response time of 7ms) which fitted snugly into the root canal directly underlying the irradiated area. Thermal measurements of the root surface were performed using a thermal camera (Inframetrix 600) with a scanning speed of 60 Hz.

Mean root surface temperatures were calculated from the maximum temperature measured during each irradiation episode. Statistical evaluation of temperature increases with regard to maximum rise between pre-irradiation and irradiation episodes was determined using the two-tailed Student's t-test.

2.3 Examination by light microscopy

All samples were examined using an Olympus SZH-III D light microscope at magnifications of up to 60X.

2.4 Preparation for SEM study

After dehydration in a graded series of aqueous ethanol (30, 50, 70, 90, 100% ethanol) for 10 minutes at each concentration, samples were mounted on a stub using colloidal silver liquid (Ted Pella, Redding, CA) with the treated areas facing upward. Specimens were gold-coated on a PAC-1 Pelco advanced coater 9500 (Ted Pella). Micrographs were taken on a Philips 515 (Mohawk, N.J.) scanning electron microscope.

3. RESULTS

The root surfaces of teeth which received laser irradiation and/or root planing did not appear altered to the naked eye. In the light microscope, root surfaces of untreated samples were uneven and debris was evident. Some parallel grooves were visible in samples which had been root planed only. These grooves were also visible in some of the specimens which had been root planed, then irradiated. Teeth which had been irradiated, irrespective of whether they had also been root planed, demonstrated no noticeable changes in the light microscope at $F \leq 1$. In samples irradiated at $F = 2.7 \text{ J/cm}^2$, E.D. = 162 J/cm^2 roughness, fissuring, cracking and some surface destruction were evident.

Using SEM, untreated samples appeared substantially different compared with root surfaces after root planing and/or laser treatment. Each control surface appeared uneven, irregular and amorphous. Remnants of subgingival plaque, clumps of deposits and swirls of fibrillar material were evident.

Root surfaces in specimens which had been root-planed only demonstrated small, probably artefactual cracks at the lateral margins of the grooves produced by hand instrumentation. A loosely-textured scale-like smear layer, sometimes with underlying grooves, was observed in all specimens. Surfaces of these specimens were often characterized by numerous depressions, which corresponded to the configuration of the dentinal tubules. A few localized deposits of debris were present as well as a small number of individual collagen fibrils. Dentinal tubule orifices were very rarely visible.

Roots which had received laser treatment only all demonstrated extensive areas of debris, plaque and fibrillar, often matted deposits. Samples which had been irradiated at $F < 1 \text{ J/cm}^2$ showed no signs of laser damage (Fig. 1).

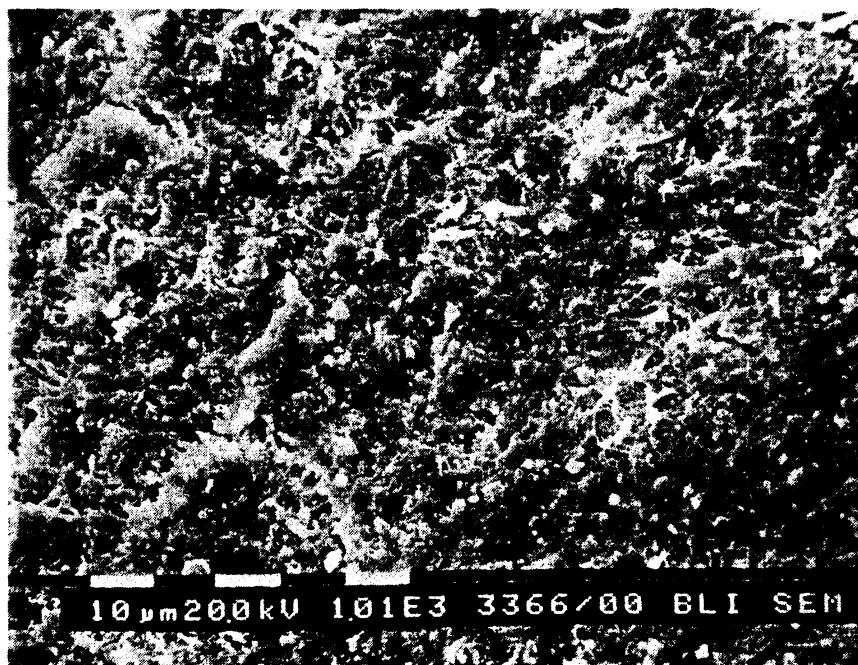


Figure 1: Root surface after laser irradiation at $F = 0.4 \text{ J/cm}^2$, 2Hz, E.D. = 12 J/cm^2 .

At parameters above this value, some patches of laser damage, showing localized small cracks and a few areas of shallow fissure-like surface destruction were widened. The higher the fluence used, the more extensive the areas of cracking, fissuring, and peeling became (Fig. 2), with patches of evident ablation.

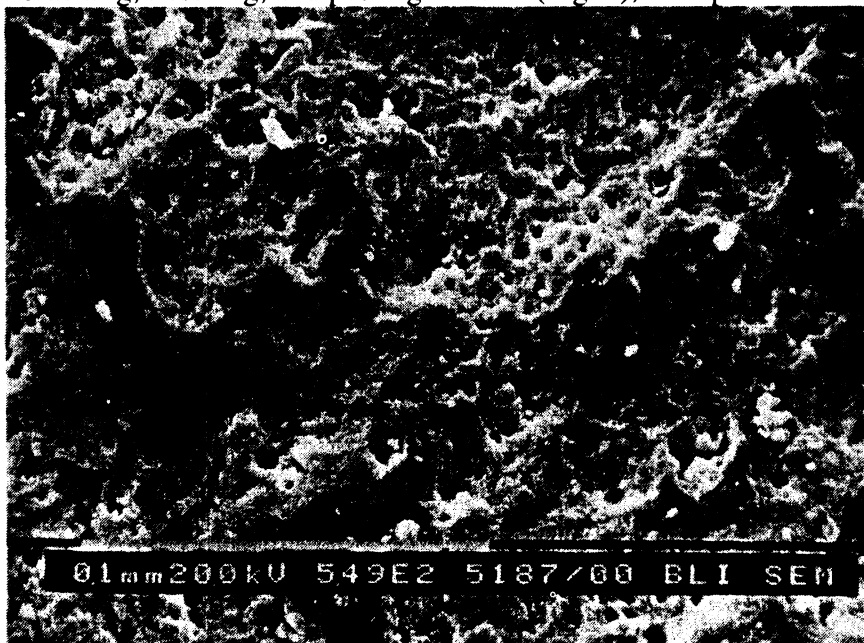


Figure 2: Root surface after laser irradiation at $F=2.7 \text{ J/cm}^2$, 2Hz, E.D.= 162 J/cm^2

In the specimens which were root planed prior to irradiation, small, probably artefactual cracks were sometimes visible at the lateral margins of the floor of the grooves produced by hand instrumentation. There was no evidence of laser damage from laser exposures of $<1 \text{ J/cm}^2$ and $< 10 \text{ Hz}$, but also little effect on the smear layer. Irradiation at $F=0.5 \text{ J/cm}^2$, 10 Hz, E.D. = 300 J/cm^2 produced localized areas free of debris with partial or occasionally complete removal of the smear layer. A few dentinal tubule orifices were visible. Specimens irradiated at $F=1.0$, 5Hz, E.D. = 75 J/cm^2 after root planing demonstrated no laser damage except a few small localized areas containing a minimal superficial microcracks and small patches of ablation. These specimens displayed many smear layer-free, relatively flat areas with some patent dentinal tubules (Fig.3).

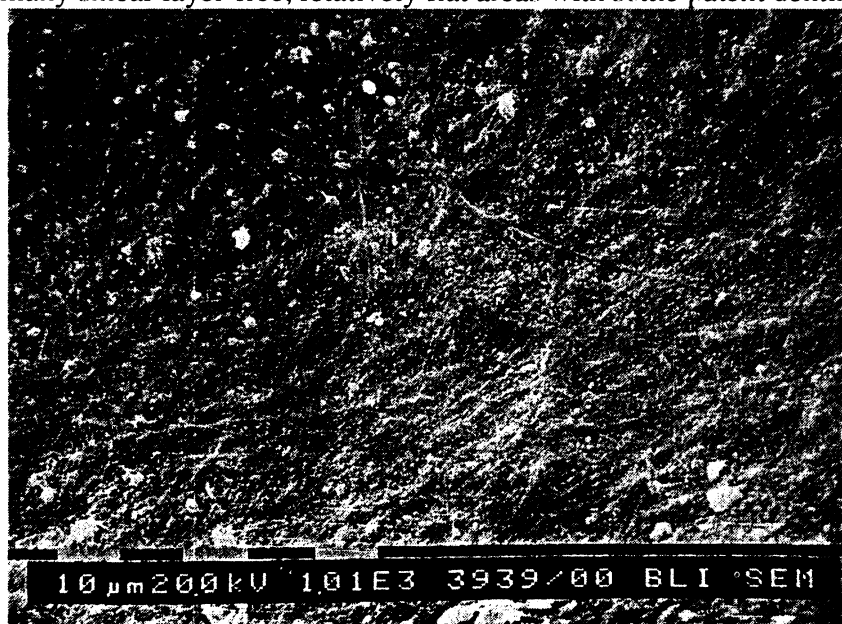


Figure 3: Root surface after root planing and laser irradiation at $F=1.0 \text{ J/cm}^2$, 5 Hz, E.D.= 75 J/cm^2

Irradiation at F=2.7 after root planing produced large areas of fissuring and cratering as well as areas of subsurface destruction and ablation.

Intrapulpal temperature increases during irradiation exceeded 5°C at $F \geq 0.5 \text{ J/cm}^2$. Recorded surface temperature rises remained below 3°C at all times (Fig. 4).

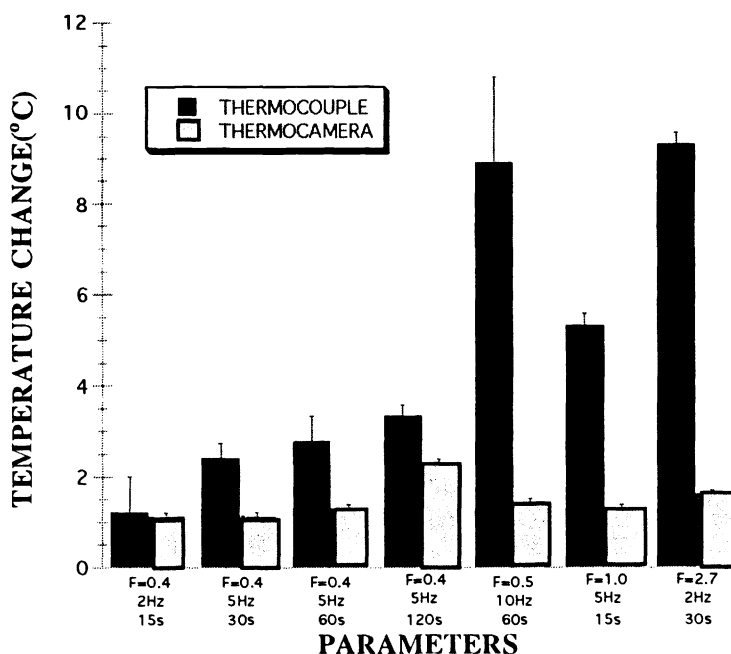


Figure 4: Root surface and intrapulpal temperature rise using short pulsed Nd: YAG laser

4. DISCUSSION

Bacterial plaque is recognized as the primary cause of periodontitis (27-32); calculus acts as a secondary etiologic factor by facilitating plaque formation and retention. Periodontally diseased root surfaces are thought to contribute to the disease process by acting as a reservoir for bacterial substances that contribute to inflammation and loss of bone and attachment (33,34). The removal of plaque, calculus and altered cementum by means of scaling and root planing is an integral part of periodontal treatment, used to obtain as clean a root surface as possible. Generally, a smear layer is seen on root-planed surfaces. Polson et al (5) suggested that root surface-connective tissue interactions will affect the biological characteristics of the smear layer, whose thickness ranges from 2 -15 microns. (17,35).

Several studies have demonstrated that citric acid conditioning can be effective for removing surface smear layer and for uncovering collagen fibers both on root-planed surfaces and within dentinal tubules (4-6,15). Root surface demineralization with citric acid has been widely accepted and adopted, and has been found to promote successfully new connective tissue attachment in both animal and human studies (11,13,14,36). Miller (37) reported that smear layer removal using citric acid produced a conditioned root surface more amenable to cell attachment and subsequent new connective tissue attachment than unconditioned root surfaces. However, bacterial invasion was observed in the dentinal tubules of citric acid-treated teeth (18) and in the superficially demineralized root surface (38). Also, exacerbation of the inflammatory pulp response and bacterial invasion was attributed to the widening of tubular apertures and smear layer resulting from the effects of citric acid (18).

Studies have suggested that topical TTC-HCl is adsorbed and actively released from root dentin (20). Following adsorption, TTC-HCl has demonstrated continued release while still maintaining bacteriostatic concentrations for most known periodontal pathogens (39). Its antimicrobial activity against subgingival microflora has produced superior clinical results. In one study, TTC-HCl irrigation resulted in significantly greater attachment gain as compared to scaling and root planing alone over a period of at least 6 months (19). However, TTC-HCl has been shown in vitro to cause surface demineralization as well as removal of the smear layer (22). Hanes et al (21) suggested that a 5% solution of TTC demineralizes the dentin tubules to a lesser degree than citric acid but that higher concentrations of TTC-HCl may be required to consistently remove the smear layer produced by instrumentation.

Local TTC-HCl delivery by fibers results in relatively high concentrations of antibiotic within the gingival crevicular fluid for up to 10 days while the fiber remains in the pocket (40). Wade et al (41) demonstrated that topical TTC-HCl appeared to cause major shifts in the composition of microflora in treated pockets, but caused a microbial selection of tetracycline-resistant organisms.

Laser therapy is being investigated for a wide range of dental applications (42). In general, CO₂ and Nd:YAG lasers are the only devices readily available specifically for clinical dental use. A few studies have been published on use of the Nd:YAG and the CO₂ laser for soft tissue procedures (42-45). However, few reports exist on laser use for any form of root debridement. Tani and Kawada (23) reported effective removal of an artificial smear layer on bovine teeth using an Nd:YAG laser at a mean output of 20W, a distance to the specimen of 3 cm and an irradiation time of 1 to 3 seconds. In a recent study by Ito et al (24) investigating the merits of Nd:YAG laser vs citric acid treatment of root surfaces, an output of 20W emitted over 0.5-3s achieved varying degrees of smear layer removal depending on the parameters used. However, the parameters utilized in these studies are not well suited to clinical application. They would tend to be associated with damage to hard tissue structures and harmful thermal effects on pulpal and periodontal vitality, especially when utilizing constant-wave irradiation. Furthermore, in a clinical situation, a total lasing duration of such brevity as described by Ito et al (24) will be impracticable. Finally, many of the lasers available for clinical use do not provide an output level of 20W.

The present study was conducted to investigate the efficacy of Q-Switched nano-second pulsed Nd:YAG laser radiation alone and after root planing for preparation of root surfaces. Laser parameters were selected to minimize the potential for thermal damage to surrounding structures.

Specimens which had been root planed exclusively demonstrated a smear layer in most areas. Ito et al (24) also observed smear layer, some debris, collagen and numerous surface depressions apparently corresponding to the opening sites of dentinal tubules on root planed surfaces.

At fluences of approximately 1 J/cm², and energy densities of 370-450J/cm², Morlock et al (25) were unable to achieve a clean root surface by Nd:YAG laser irradiation followed by root planing. A scaly smear layer was observed resembling closely the smear layer apparent after root planing only. Thus, the smear layer resulting from root planing is not affected by previous laser irradiation at these parameters.

Surfaces of specimens which had been irradiated only were covered in various deposits. Thus irradiation alone does not provide adequate debridement of the root surface. Furthermore, irradiation at or above F=1.0J/cm² were found to damage the root surface, the degree of damage increasing with greater exposure. Morlock et al (25) also described ineffective removal of root surface deposits using Nd:YAG laser irradiation alone. The surface damage they report at energy densities of approximately 350-450 J/cm² exceeds the extent of structural changes we observed, no doubt due to the higher fluences and frequencies and longer pulse durations they employed (0.9, 1.1 J/cm², 20 Hz).

Results achieved in samples irradiated after root planing were directly related to the fluences and energy densities utilized. Removal of smear layer was evident in samples irradiated at F=1.0 J/cm². Here, good results were achieved without microstructural evidence of thermal damage at energy densities approximating 75 J/cm². However, consistent results could not be obtained. Also, intrapulpal temperature rises measured during irradiation generate concerns regarding possible pulp damage. Ito et al (24) also achieved effective removal of surface smear layer after root planing using the constant-wave Nd:YAG laser at energy densities of approximately 85-850 J/cm². However, in an unpublished study they found that these effects were accompanied by temperature rises of up to 115 C, despite the reported absence of any microstructural

manifestations of thermal damage to adjacent hard dental tissues. Certainly temperature increases of this magnitude would give rise to concerns regarding detrimental pulpal and periodontal effects.

Once fluences exceeded 1.0 J/cm², a progressive amount of structural damage became apparent in our specimens. The cracking and fissuring observed is thought to be related to increasing temperature rises associated with longer exposure times. Hence there exists some cause for concern regarding thermally-induced pulpal and periodontal damage. Moreover, ablation also occurred at higher fluences. Using the Nd:YAG laser at higher energy densities, Myers (42,46) also reported fissuring and pitting, ranging in depth from 40 - 60µ, carbonization and charring as well as melting of root surface structures. In an in vivo study, Cobb et al (26) observed some good, if inconsistent, results using a pulsed Nd:YAG laser at energy densities of approximately 105 J/cm² after root planing, but laser-induced root surface alterations occurred at energy densities of 400-540 J/cm². The relative severity of the thermal effects determined in their investigation as opposed to ours is probably related to their use of a longer pulse and a frequency of 20 Hz, as opposed to the 5 Hz used in our study. This concept is reinforced by the absence of thermal damage observed by Cobb et al when they used shorter irradiation times. Additionally, their investigation was carried out in vivo, working within the periodontal pocket, which would reinforce heat retention.

In conclusion, the results of this study indicate that root surface smear layer can be removed using non-contact delivery of nano-second pulsed Q-Switched Nd:YAG laser irradiation. However, achieving consistent, evenly distributed results was problematic and thermal events dictate caution.

5. ACKNOWLEDGEMENTS

The authors would like to thank TJ Desai and Tri Phan for their assistance with sample preparation for SEM and Elizabeth Berry for preparation of this manuscript.

This study was supported by DOE grant DE 903-91ER 61227, ONR grant N00014-90-0-0029 and NIH grant RRO1192.

6. REFERENCES

1. Jones W, O'Leary T. "The effectiveness of in vivo root planning in removing bacterial endotoxin from roots of periodontally involved teeth." *J Periodontol*. 49:337-342, 1979.
2. Daly C, Seymour J, Kieser J. Bacterial endotoxin: A role in chronic inflammatory periodontal disease. *J Oral Pathol* 198-9;1-15, 1983.
3. Jones S, Lozdan J, Boyde A. "Tooth surfaces treated in situ periodontal instruments." SEM studies. *Br Dent J* 132:57-64, 1972.
4. Lasho DJ, O'Leary TJ, Kafrawy AH. "A scanning electron microscopic study of the effects of various agents on instrumented periodontally involved root surfaces." *J Periodontol* 54:210-220, 1983.
5. Polson AM, Frederic GT, Ladenheim S, Hanes PJ. "The production of a root surface smear layer by instrumentation and its removal by citric acid". *J Periodontol* 55:443-446, 1984.
6. Ito K, Sugano N, Nishikata J, Ujiie H, Fujikawa K, Murai S. "Effects of various agents to remove smear layers on root planed periodontally involved root surfaces." In Gold SI, Midda M, Mutla S, eds. Recent Advances in Periodontology, Vol. II. Amsterdam: Elsevier Science Publishers BV; 475-478, 1991.
7. Kerns, DG, Scheidt MJ, Pashley DH, Horner JA, Strong SL, Van Dyke TE. "Dentinal tubule occlusion and root hypersensitivity." *J Periodontol* 62:421-428, 1991.
8. Hatfield CG, Baumhammers A. "Cytotoxic effects of periodontally involved surfaces of human teeth." *Arch Oral Biol*, 6:465-468, 1971.
9. Aleo JJ, DeRenzis FA, Farber PA, Varboncoeur AP. "The presence and biologic activity of cementum-bound endotoxin." *J Periodontol*, 45:672-675, 1974.
10. Nishimine D, O'Leary TJ. "Hand instrumentation versus ultrasonics in the removal of endotoxins from root surfaces." *J Periodontol*, 50:345-349, 1979.
11. Albair WB, Cobb CM, Kilroy WJ "Connective tissue attachment to periodontically diseased roots after citric acid demineralization". *J Periodontol*, 53:515-526, 1982.

12. Sarbinoff JA, O'Leary TJ, Miller CH. "The comparative effectiveness of various agents in detoxifying diseased root surfaces", *J Periodontol*, 54:77-80, 1983.
13. Lopez NJ. "Connective tissue to periodontally diseased roots, planed and conditioned with citric acid implants into the oral mucosa." *J Periodontol* , 55:381-390, 1984.
14. Smith BA, Mason WE, Morrison EC, Caffesse RG. "The effectiveness of citric acid as an adjunct to surgical reattachment procedures in humans." *J Clin Periodontol*, 13:701-708, 1986.
15. Sterrett J, Merphy H. "Citric acid burnishing of dentinal root surfaces. A scanning electron microscopy report." *J. Clin Periodontol*, 16:98-104, 1989.
16. Polson AM, Frederick GT, Ladenheim S, Hanes P. "The production of a root surface smear layer by instrumentation and its removal by citric acid." *J Periodontol* , 55:443-46, 1984.
17. Brannstrom M, Johnson G. "Effects of various conditioners and cleaning agents on prepared dentin surfaces. A SEM investigation." *J Prosthet Dent* , 31:422-430, 1974.
18. Ryan PC, Newcomb GM, Seymour GJ, Power RN. "The pulpal response to citric acid in cats." *J Clin Periodontol*, 11:633-643, 1984.
19. Christersson LA, Norderyd OM and Puchalsky CS. "Topical application of tetracycline-HCl in human periodontitis." *J Clin Periodontol*, 20:88-95, 1993.
20. Wilkesjo UME, Baker PJ, Christersson LA, Genco RJ, Lyall RM, Hic, S. et al. "A biochemical approach to periodontal regeneration: Tetracycline treatment conditions dentin surfaces." *Journal of Periodontal Research*, 21,322-329, 1986.
21. Hanes PJ, O'Brien NJ and Garnick JJ: "A morphological comparison of radicular dentin following root planing and treatment with citric acid or tetracycline HCL." *J Clin Periodontol*, 18:660-668, 1991.
22. Adreaens PA, Edwards CA, DeBoever JA, Loescne WJ, Ultrastructural observations on bacterial invasion in cementum and radicular dentin of periodontally diseased human teeth. *J Periodontol*, 59:493-503, 1988.
23. Tani Y, Kawada H. "Effects of laser irradiation on dentin. I. Effect on smear layer." *Dent Materials*. J, 6:127-134, 1987.
24. Ito K, Nishikata T, Murai S. Effects of Nd:YAG laser radiation on removal of root surface smear layer. *J Periodontol*, 64:547-552, 1993.
25. Morlock BJ, Pippin DJ, Cobb CM et al. "The Effect of Nd:YAG Laser Exposure on Root Surfaces When Used as an Adjunct to Root Planing: An In Vitro Study." *J Periodontol*, 63:637-41, 1992.
26. Cobb M, McCawley TK, Killoy WJ. "A preliminary study on the effects of the Nd:YAG laser on root surfaces and subgingival microflora in vivo." *J Periodontol*, 63:701-7, 1992.
27. Loe H, Theilade E, Jensen S, Schiott C. "Experimental gingivitis in man. III. The influence of antibiotics on gingival plaque development." *J Periodont Res*, 2:282-289, 1967.
28. Page R, Schroeder H. "Pathogenesis of inflammatory periodontal disease - A summary of current work." *Lab Invest*, 33:235-249, 1976.
29. Theilade E, Wright W, Jensen S, Loe H. "Experimental gingivitis in man II. A longitudinal clinical and bacteriologic investigation." *J. Periodont Res*, 1:1-13, 1966.
30. Loe H. "Human research model for the production and prevention of gingivitis." *J Dent Res*, 50:256-264, 1971.
31. Socransky S. "Relationship of bacteria to the etiology of periodontal disease." *J Dent Res*, 49:203-22, 1970.
32. Loe H. Theilade E, Jensen S. "Experimental gingivitis in man." *J Periodontol*, 36:177-187, 1965.
33. Jones W, O'Leary T. "The effectiveness of in vivo root planing in removing bacterial endotoxin from roots of periodontally involved teeth." *J Periodontol*, 49:337-342, 1978.
34. Daly C, Seymour J, Kieser J. "Bacterial endotoxin: A role in chronic inflammatory periodontal disease." *J Oral Pathol* , 9:1-15, 1980.
35. Eick JD, Wilko RA, Anderson CH, Sorensen SE. "Scanning electron microscopy of cut tooth surfaces and identification of debris by use of the electron microprobe." *J Dent Res*. 49:1359-1368, 1970.
36. Cole RT, Crigger M, Bogle G, Egelberg J, Selvig KA. "Connective tissue regeneration to periodontally diseased teeth. A histological study." *J Periodont Res*,15:1-9, 1980.

37. Miller PD. "Root coverage using the free soft tissue autograft following citric acid application. III. A successful and predictable procedure in areas of deep-wide recession." *Int J Periodontics Restorative Dent* 5(2):15-37, 1985.
38. Langeland K, Rodriguez H, Dowden W. "Periodontal disease, bacteria and pulpal histopathology." *Oral Surg*, 37:257-270, 1974.
39. Baker PJ, Evans RT, Coburn RA and Genco RJ. "Tetracycline and its derivatives strongly bind to and are released from the tooth surface in active form." *J of Periodontology* , 54:580-85, 1983.
40. Goodson JM, Holborow D, Dunn RL, Hogan P and Dunham S. "Monolithic tetracycline-containing fibers for controlled delivery to periodontal pockets." *J of Periodontology* , 56 (spec. iss.) 81-7, 1983.
41. Wade WG, Moran J, Morgan JR, Newcombe R and Addy M. "The effects of antimicrobial acrylic strips on the subgingival microflora in chronic periodontitis." *J Clin Periodontol*, 19:127-134, 1992.
42. Myers TD. "Lasers in dentistry". *J AM Dent Assoc* , 122(1):47-50, 1991.
43. Pick RM, Pecaro BC, Silberman CT. "The laser gingivectomy. The use of the CO2 laser for the removal of phenytoin hyperplasia." *J Periodontol*, 56:492-496, 1985.
44. Rossmann JA, Gottlieb S, Kondelka BM, McQuade MJ. "Effects of CO2 laser irradiation on gingiva." *J Periodontol*, 58:423-425, 1987.
45. Barak S, Kaplan I. "The CO2 laser in the excision of gingival hyperplasia caused by nifedipine." *J Clin Periodontol* , 15:633-635, 1988.
46. Myers TD. "Effects of a pulsed Nd:YAG laser on enamel and dentin." In: Joffe SN, Atsumi K, eds. *Proceedings of Laser Surgery: Advanced Characterization, Therapeutics, and Systems II*. Bellingham, WA: SPIE-International Society for Optical Engineering, 425-436, 1990.