Lasers Med Sci
DOI 10.1007/s10103-007-0482-6

ORIGINAL ARTICLE

Nd:YAG laser clinical assisted in class II furcation treatment

Ana Karina Pinto de Andrade · Ilíria Salomão Feist · Cláudio Mendes Pannuti · Silvana Cai · Denise Maria Zezell · Giorgio De Micheli

Received: 22 February 2007 / Accepted: 30 May 2007

Abstract The Nd:YAG laser efficacy associated with conventional treatment for bacterial reduction has been investigated throughout literature. The purpose of this study was to evaluate the bacterial reduction after Nd:YAG laser irradiation associated with scaling and root planning in class II furcation defects in patients with chronic periodontitis. Thirty-four furcation lesions were selected from 17 subjects. The control group received conventional treatment, and the experimental group received the same treatment followed by Nd:YAG laser irradiation (100 mJ/pulse; 15 Hz; 1.5 W, 60 s, 141.5 J/cm^2). Both treatments resulted in improvements of most clinical parameters. A significant reduction of colony forming unit (CFU) of total bacteria number was observed in both groups. The highest reduction was noted in the experimental group immediately after the treatment. The number of dark pigmented bacteria and the percentage of patients with *Porphyromonas gingivalis*, *Prevotella intermedia*, and *Actinobacillus actinomycetemcomitans* reduced immediately after the treatment and returned to values close to the initial ones 6 weeks after the baseline for both groups. The Nd:YAG laser associated with conventional treatment promoted significant bacterial reduction in class II furcation immediately after irradiation, although this reduction was not observed 6 weeks after the baseline.

Keywords Periodontal diseases/therapy · Bacterial reduction · Nd:YAG laser

A. K. P. de Andrade (✉) · I. S. Feist · C. M. Pannuti · G. De Micheli
Stomatology Department, Periodontics Discipline,
School of Dentistry, University of São Paulo (USP),
Av Prof Lineu Prestes 2227, Cep 05508-900 Cidade Universitária,
São Paulo, Brazil
e-mail: akpandrade@yahoo.com

I. S. Feist
e-mail: iliria.ops@terra.com.br

C. M. Pannuti
e-mail: pannuti@usp.br

G. De Micheli
e-mail: gmicheli@plugnet.com.br

S. Cai
Microbiology Department of the Institute of Biomedical Sciences,
University of São Paulo (USP),
Av Prof Lineu Prestes 1374 Ed. Biomédicas II, Cep 05508-900.
Cidade Universitária,
São Paulo, Brazil
e-mail: silvanac@icb.usp.br

D. M. Zezell
Centro de Lasers e Aplicações,
Instituto de Requisas Energéticas e Nucleares–IPEN/CNEN-SP,
Av Prof Lineu Prestes 2242, Cep 05508-000 Cidade Universitária,
São Paulo, Brazil
e-mail: zezell@usp.br

Introduction

During the last few years, the laser technology has been used as a main or coadjutant tool on the several dental areas. The studies began with Einstein in 1917 when he mathematically postulated that portions of the electromagnetic field could be stimulated and emit amplified light. The first use of laser in dentistry took place with Stern and Sognnaes [1] vaporizing human enamel and dentin through the use of a ruby laser.

Neodymium laser was developed by Geusic et al. [2] but it was only in 1998 when it achieved its approval by the Food and Drugs Administration (FDA) to be used in the laser curettage procedures where this light beam is applied

inside the periodontal pockets in a contact mode with the soft tissue aiming for bacterial reduction [3].

The Nd:YAG laser was the first wavelength developed exclusively for dentistry [4]. This laser operates on a pulsed mode, with 150 μs pulse duration, causing thus less thermal damage when compared with lasers operating on a continuous mode [5]. It emits a wavelength of 1,064 nm being delivered through an optical fiber system with a diameter varying from 200 to 800 μm, which is resonant with absorption of cellular elements and mainly with pigments such as hemoglobin [6].

Periodontal diseases result from infections originated by the bacterial biofilm particularly by Gram-negative anaerobic bacteria [7]. The root management associated with an adequate plaque control is focused on the periodontal therapy being a fundamental resource and widely applied on the pathology control.

Multi-rooted teeth with furcation involvement represent a significant challenge for the periodontal treatment mainly due to the irregularity and complex anatomy of this particular region. This enables the plaque accumulation and hinders the access for an adequate instrumentation of the root surface and also for the hygiene process by the patient [8] leading to a limited treatment outcome [9].

Thus, the purpose of this present study was to use the Nd:YAG laser in furcation defects associated with the conventional periodontal therapy aiming the evaluation of the possibility of periodontal pathogens reduction in periodontal pockets and optimization of clinical parameters derived from this treatment.

Materials and methods

Study design and population

The experimental protocol consisted of a double-blind, randomized, clinical trial where the patients with chronic periodontal disease [10] were selected at the Periodontics Clinic at the School of Dentistry of the University of São Paulo. Seventeen patients were treated with furcations randomly assigned to one of the two experimental groups: (1) scaling and root planning (control site) and (2) pulsed Nd:YAG dental laser as an adjunct to standard treatment after scaling and root planning (test site).

The patients presented class II furcation lesions [11] in two vital teeth with clinical probing depth of more than 4 mm for the vertical component and equal or more than 6 mm for the furcation horizontal component without any previous periodontal treatment or antibiotic therapy within 6 months before this study. The probing procedure was made using an acrylic guide and a PCPUNC 15 North Carolina probing instrument (Hu-Friedy–USA).

This research was approved by the experimental Ethical Committee of the School of Dentistry at the University of São Paulo according to the 153/02 process.

Patient preparation and inclusion

A calibrated examiner 1 conducted the anamnesis, periodontal chart and X-ray procedures. The patients also received oral hygiene orientation and were submitted to the first clinical measurements and microbiological data collection (baseline). All the patients received previous periodontal treatment up to four sessions with the exception of the molars selected for this study, which were treated during the experimental phase.

The furcation selected for this study went through ultrasonic instrumentation (EMS, Germany) into vertical and horizontal components followed by a scaling and root planning procedure with Gracey 11/12, 13/14, and McCall 13/14 curettes (Hu-Friedy, USA) on the control side. The same treatment was performed on the experimental side followed by Nd:YAG laser irradiation. This procedure was repeated after 1 week in both control and test sites. A second subgingival biofilm collection (moment 1) was made immediately after the end of the treatment. The second clinical measurements and the third biofilm collection of the interior of the periodontal pocket were accomplished 6 weeks after the baseline (moment 2).

Nd:YAG laser irradiation

The experimental and control sites were randomly determined by the examiner 2 by the flip of a coin. The laser energy was measured before each irradiation by a pyroelectric detector LM10i connected to a power/energy meter Fieldmaster (Coherent, Auburn, USA). The Nd:YAG laser (ADT–American Dental Technology, USA) irradiation parameters at the experimental sites were: 100 mJ/pulse, 1.5 W mean power; 15 Hz repetition rate, with pulse duration of 150 μs The energy density at the end of the fiber was 141.5 J/cm^2. The 300-μm diameter optical fiber tip was placed parallel to the teeth surface (vertical component) at the furcation area being brushed over this treatment area with light contact against the pocket epithelium for 30 s. The fiber was kept in constant motion, moving yet in a sweeping direction starting at 1 mm from the clinical probing depth [12] and progressing in a coronal direction. The fiber was also applied at 90° at the horizontal component with circular movements for 30 s totalizing a time of 60 s per furcation in each irradiation session. The pulp vitality in all treatment sites was evaluated through thermal tests and continually monitored for changes before the experiment and 6 weeks post-treatment.

Table 1 Mean, medium, and standard deviation of the experimental groups test (T) and control (C) regarding the gingival margin cement enamel junction distance (GM-ECJ), clinical probing depth of the furcation vertical component (PDV), and clinical attachment level (CAL) at the different moments

Moments	GM-ECJ T	GM-ECJ C	*p* value[a]	PDV T	PDV C	*p* value[a]	CAL T	CAL C	*p* value[a]
Baseline									
Mean	3.3	2.7	0.24	4.9	4.8	0.79	8.1	7.6	0.48
Medium	3.0	3.0		4.0	4.0		8.0	8.0	
SD[b]	1.5	1.3		1.3	1.3		2.1	1.5	
6 weeks after									
Mean	3.6	3.5	0.79	3.1	2.9	0.70	7.1	6.0	0.13
Medium	4.0	3.0		3.0	3.0		7.0	6.0	
SD[b]	1.6	1.8		1.1	1.0		2.6	1.9	
p value[c]	0.21	0.06		<0.001[d]	<0.001[d]		0.005[d]	<0.001[d]	

[a] Between beginning and 6 weeks
[b] *DP* standard deviation.
[c] Between test and control group
[d] Statistical significance at 5%

Clinical evaluation

The inflammation level was measured through the gingival and plaque indices [13, 14], the clinical probing depth of the furcation vertical and horizontal components besides the clinical attachment level were also evaluated. All these measures were recorded by the examiner 1 who was blind about the site treated with the Nd:YAG laser.

Microbiological evaluation

The teeth were isolated with cotton rolls, and the supragingival plaque was removed with periodontal curettes and two sterilized no. 40 paper points were introduced inside the periodontal pockets for 30 s for the biofilm collection [15]. Following this, the points were placed in a vial containing 3 ml of the transport media (VGMA III) [16] with glass beads. The samples were processed 24 h after the sample collection.

The vials containing the points in the transport media were incubated at 37°C for 30 min, and the samples were homogenized (Fisher Vortex Genie 2, USA). Amounts of 100 μl from non-diluted and diluted samples at 1/10 and 1/100 in peptone water were sampled in Petri plates containing a selective tryptic soy-serum-bacitracin-vancomycin (TSBV) culture media [17]. The presumptive identification of *A. actinomycetemcomitans* was made 3 days after the incubation period at 37°C in a 10% of CO_2 atmosphere. The identification was based on the colony morphologic aspect using a stereoscopic microscope; Gram-staining, and catalase test.

Aliquots of 100 μl from each sample, diluted to 1/100, 1/1,000 and 1/10,000, in peptone water were plated on Brucella agar with 5% of defibrinated sheep blood, hemine (10 μg/ml, Sigma H-2250) and menadione (1 μg/ml, Sigma

Table 2 Mean, medium, and standard deviation of the experimental groups test (T) and control (C) regarding the Plaque Index (PI), Gingival Index (GI), and Clinical Probing Depth of the furcation horizontal component (PDH) at the different moments

Moments	PI T	PI C	*p* value[a]	GI T	GI C	*p* value[a]	PDH T	PDH C	*p* value[a]
Baseline									
Mean	2.11	2.29	0.38	2.17	2.17	1.00	6.9	6.9	1.00
Medium	2.00	2.00		2.00	2.00		6.0	6.0	
SD[b]	0.48	0.77		0.52	0.63		1.4	2.0	
6 weeks after									
Mean	1.00	1.17	0.33	1.11	0.88	0.21	6.3	6.0	0.54
Medium	1.00	1.00		1.00	1.00		6.0	6.0	
SD[b]	0.61	0.88		0.85	0.69		1.8	1.8	
P value[c]	<0.001[d]	<0.001[d]		<0.001[d]	<0.001[d]		0.08	0.056	

[a] Between beginning and 6 weeks
[b] *DP* Standard deviation
[c] Between test and control group
[d] Statistical significance at 5%

Table 3 Mean, medium, and standard deviation of the experimental groups test (T) and control (C) regarding the colony forming units (CFU) of total bacteria number at different moments

Moments	CFU of total bacteria number T	CFU of total bacteria number C	*p* value[a]
Baseline			
Mean	3.34×10^5	2.80×10^5	0.65
Medium	2.30×10^5	1.48×10^5	
SD[b]	2.66×10^5	3.67×10^5	
Immediately after			
Average	1.86×10^4	3.14×10^4	0.019[c]
Medium	7.85×10^3	7.00×10^3	
SD[b]	2.43×10^4	3.71×10^4	
6 weeks after			
Average	1.11×10^5	1.42×10^5	0.37
Medium	6.65×10^4	8.70×10^4	
SD[b]	1.51×10^5	1.86×10^5	
p value[d]	<0.0001[c]	<0.0001[c]	

[a] Between baseline, immediately after and 6 weeks
[b] *DP* Standard deviation.
[c] Statistical significance at 5%
[d] Between test and control group

M-5625) [18]. After 7 days of incubation at 37°C in an anaerobic atmosphere (Anaerobic Jar 2.5 L, Oxoid), the microbial indices were monitored and recorded regarding the number of CFU of total and dark- pigmented bacteria was made.

The dark-pigmented bacteria colonies were presumptively identified based on the lactose fermentation test [19] using the 4-metylumbeliferril-β-D-galactosidase substrate (MUG; M-1633, Sigma), at a concentration of 1% in dimethylsulfoxide (D-8799, Sigma). The trypsin activity test [20] was made using the fluorogenetic carbobenzoxy L-arginine-7-amino-4metylcoumarine amine-HCL (CAAM) synthetic compound. The colonies with positive autofluorescence when submitted to a long ultraviolet light wavelength (365 nm Mineralight Lamp, U.V.G.L.–58, USA), MUG, and CAAM-negative test [19] were identified as *P. intermedia*, and the ones which presented negative autofluorescence, negative MUG test, and positive CAAM test were identified as *P. gingivalis* [17].

Statistical analysis

The Levene test was used to verify the variance homogeneity, while the distribution normality was verified by the Kolmogorov–Smirnov test. The Student's paired *t* test for the related samples was used to verify differences among the experimental and control groups.

In the cases where no adherence was observed on the normal curve or where the variances were not homogeneous, the non-parametric Wilcoxon statistical test was applied. The changes along the time were evaluated either through the *t* test or through the Wilcoxon test.

Regarding the microbiological variables measured in three moments, the non-parametric Friedman statistical test was used to examine the possible changes along the time. The Dunn statistical test was used to indicate evidences between which moments the differences were present.

The chi-square or the Fisher statistical tests were used to verify if any association between the experimental group and presence of studied bacteria was observed.

The statistical analysis was performed through SPSS software for Windows (version 5.2) with a significance level of 5% in all the statistical tests.

Results

Seventeen subjects—nine females (52.9%) and eight males (47.1%)—of a mean of 47.4 years were evaluated. Twelve subjects (70.6%) were no smoking patients. Both groups represented an increase of the gingival margin cement enamel junction (GM-CEJ) being this value of 0.3 mm for the experimental site and 0.8 mm for the control site both without any statistically significant difference regarding the groups and moments analyzed in this study (Table 1).

It could be observed that there was a mean significant decrease of 1.8 mm ($p<0.001$) for the experimental site and 1.9 mm ($p<0.001$) for the control site with no statistically

Table 4 Mean, medium, and standard deviation of the experimental groups test (T) and control (C) regarding the total colony forming units (CFU) of dark-pigmented bacteria at the different moments

Moments	Total CFU of dark-pigmented bacteria T	Total CFU of dark-pigmented bacteria C	*p* value[a]
Baseline			
Mean	5.93×10^3	6.71×10^3	0.87
Medium	5×10^2	3.30×10^3	
SD[b]	15.37×10^3	9.68×10^3	
Immediately after			
Mean	1.17×10^2	1.04×10^3	0.27
Medium	0.00	0.00	
SD[b]	4.85×10^2	2.83×10^3	
6 weeks after			
Mean	5.78×10^3	9.85×10^3	0.16
Medium	1.20×10^3	4.00×10^2	
SD[b]	13.84×10^3	19.44×10^3	
p value[c]	0.002[d]	0.013[d]	

[a] Between baseline, immediately after and six weeks
[b] *DP* Standard deviation
[c] Between test and control group
[d] Statistical significance at 5%

Table 5 Percentage values of patients with *P. gingivalis*, *P. Intermedia*, and *A. Actinomycetemcomitans* on the experimental groups test (T) and control (C) at different moments

Moments	*P gingivalis* (%)	*p* value[a]	*P. intermedia* (%)	*p* value[a]	*A. Actinomycetemcomitans* (%)	*p* value[a]
Baseline						
T	41.2	0.49	17.6	0.43	23.5	0.65
C	52.9		35.3		11.8	
Immediately after						
T	5.9	1.00	0.00	0.48	0.00	1.00
C	5.9		11.8		5.9	
6 weeks after						
T	29.4	0.62	23.5	1.00	11.5	1.00
C	37.5		23.5		17.6	

[a] Between baseline, immediately after and 6 weeks after

significant difference between the average values of both sites at the beginning (p=0.79) or after 6 weeks of baseline (p=0.70) when the clinical probing depth of the furcation vertical component was evaluated (Table 1).

A significant reduction of the clinical attachment level was observed on the experimental (p=0.005) and on the control (p<0.001) sites without any difference between then along the study (Table 1).

Regarding the clinical probing depth of the furcation horizontal component, it was possible to observe that there was a mean reduction of 0.6 mm on the experimental site, but no difference between the initial and final measure (p=0.08) was observed. There was a mean reduction of 0.9 mm on the control sites, but this difference is also not statistically significant (p=0.056). There was no significant difference between the average values of both sites considering the initial (p=1.00) and final (p=0.54) values (Table 2).

A significant reduction of the plaque index and gingival index was observed on the experimental (p<0.001) and control groups (p<0.001). On the other hand, no difference could be detected between both sites regarding this variable in any moment of the study (Table 2).

There was no significant difference regarding the CFU total bacteria number between the two sites before (p=0.65) and 6 weeks after the baseline (p=0.37). However, a significant difference between the sites immediately after the treatment (p=0.019) was observed where the experimental group presented statistically higher reduction when compared with the control site (Table 3).

No significant difference was observed between the sites before (p=0.87) and 6 weeks after the baseline (p=0.16) regarding the total number of dark-pigmented bacteria. The average number of CFU dark-pigmented bacteria was higher on the control site than on the experimental site immediately after the treatment, although there was not any statistically difference between than (p=0.27) (Table 4).

The *P. gingivalis*, *P. intermedia*, and *A. actinomycetemcomitans* analysis according to the presence or absence on the experimental groups showed no association between the experimental group and the presence of the studied bacteria in any moment of this present research (Table 5).

Discussion

Studies with Nd:YAG laser using different parameters [21, 22] showed that mean power settings ranging between 1.25 and 3 W promote a root surface change leading to fusion and resolidification of the cement mineral portion besides cracks and fissures formation. Radvar and McFarlane [23] and Thomas et al. [24] suggested that the complement of the conventional mechanical treatment of the root surface would be important on the reduction of these irregularities aiming for a more biocompatible root surface [23]. Hatit et al. [12] demonstrated that several irradiations are necessary for these changes to cause a relevant clinical effect.

Although Chan and Chien [25] had demonstrated that the laser irradiation with mean power higher than 3 W were effective on the bacterial reduction and that Pinero [26] demonstrated the laser efficacy in mean power settings varying from 5 to 10 W associated with the conventional treatment regarding the subacute bacterial endocarditic prevention, it is important to consider the more adequate parameter to preserve the root and adjacent tissues integrity.

The laser parameters used in this study (100 mJ/pulse, 15 Hz, 1.5 W) were based on some literature studies [12, 22, 27], which demonstrated that the Nd:YAG laser when used with mean power up to 2 W promoted bacterial reduction with minimum root surface hazard effects.

The results observed in the present study agree with Lin et al. [27], Neil and Melonig [28], and Gutknecht et al. [3] in which laser associated with conventional treatment have not demonstrated any additional clinical benefit when compared with root scaling and planing alone regarding the clinical probing depth, clinical attachment level and retraction degree. However, according to Horton and Lin

[29], the clinical probing depth was the same or a better result was achieved for the group where the laser was used.

Neil and Melonig [29], using 120 mJ/pulse, 0.8 W, and 20 Hz, demonstrated that the group where the laser was associated with root scaling and planing presented better results when compared with the control group. These results disagree with our findings where no statistical significant differences between the groups were observed.

Some in vitro studies demonstrated the bacterial reduction after the Nd:YAG laser irradiation [6, 24, 30] and associated with the periodontal therapy [3, 12, 22, 26, 31]. However, no pattern regarding the energy and time parameters applied for bacterial reduction on the periodontal pockets were observed throughout the literature papers, which hinders a more precise analysis of the results and also an adequate comparison among the studies.

In this study, 34 teeth with degree II furcation involvement were treated only with conventional periodontal therapy or with root scaling and planing associated with the Nd:YAG laser (100 mJ/pulse, 15 Hz, 1.5 W, 60 s). The results demonstrated that the association with the laser promoted a significant higher reduction on the total bacteria CFU number, but after 6 weeks, this number was higher again in both groups being in accordance with the results observed by Sbodone et al. [32].

Hatit et al. [12] and Gutknecht et al. [3] demonstrated that the nondetection of *A. actinomycetemcomitans* was not possible either after the conventional treatment or after the root scaling and planing associated with the Nd:YAG laser irradiation. The same situation was observed in the present study. According to Hatit et al. [12], the difficulty in eliminating this microorganism lies on the invasion of the gingival tissues by them both in patients with aggressive periodontitis and chronic disease. This studies [3, 12] demonstrated although a higher *A. actinomycetemcomitans* reduction where there was laser irradiation when compared with the conventional treatment, which was not observed in this study where no significant difference was observed among the groups regarding these microorganisms reduction.

It is possible to observe that some research papers [3, 12] demonstrate through a DNA probe a superior *P. gingivalis* and *P. intermedia* reduction where the laser was associated with the conventional treatment after 30 days of treatment. Neil and Melonig [28] related through the same microbiological diagnosis method that there was a reduction on the number of these bacteria after the treatment but with no difference among the groups (root scaling and planing isolated and root scaling associated with the laser irradiation). Similar results to this study were obtained in the present study [28].

Certainly the search for answers regarding the laser action when applied in the periodontal tissues where different tissues interact in a dynamic way represents a challenge in modern dentistry. This suggests further studies to establish adequate parameters and/or number of sessions for the initial bacterial reduction achieved in this study to be kept for a longer period of time.

Conclusions

According to the methodology developed in this clinical study, these findings suggest that the Nd:YAG laser associated with the root scaling and planing promoted reduction of a mean number of CFU of total bacteria number when compared with the control group immediately after its application. The Nd:YAG laser irradiation associated with root scaling and planing and the isolated conventional periodontal treatment demonstrated statistically significant increased clinical conditions with these parameters 6 weeks after the treatment, but no statistically significant differences were observed between the groups.

Acknowledgements The authors would like to thank Fundação de Amparo a Pesquisa no Estado de São Paulo–FAPESP, research grants # 2000/90236-7, # 05/051689-2, Procad/CAPES and Instituto Milenio Fotonica/CNPq.

References

1. Stern RH, Sognnaes RF (1964) Laser beam effect on dental hard tissue. J Dent Res 43(5):873
2. Geusic JE, Marcos HW, Van Uitert LLG (1964) Laser oscillations in Nd-doped yttrium aluminum, yttrium gallium, and gadolinium garnets. Appl Phys Lett 4:182
3. Gutknecht N, Raoufi P, Franzen R, Lamper F (2002) Reduction of specific microorganisms in periodontal pockets with the aid of an Nd:YAG laser: an *in vivo* study. J Oral Laser Appl 3:175–180
4. Coluzzi DJ (2000) An overview of laser wavelengths used in dentistry. Dent Clin North Am 44(4):753–765
5. Pick RM (1997) The use of the laser for treatment of gingival diseases. Oral Maxillofac Surg Clin North Am 9(1):6–7
6. White JM, Goodis HE, Setcos JC, Eakle S, Hulscher BE, Rose CL (1993) Effects of pulsed Nd:YAG laser energy on human teeth: a three-year follow-up study. Am Dent Assoc 124(58):45–51
7. Loesche WJ (1992) The specific plaque hypothesis and the antimicrobial treatment of periodontal disease. Dent Update 19 (2):68–74
8. Al-Shammari KF, Kazor CE, Wang H-L (2001) Molar root anatomy and management of furcation defects. J Clin Periodontol 28:730–740
9. Cattabriga M, Pedrazzoli V, Wilson TG Jr (2000) The conservative approach in the treatment of furcation lesions. Periodontology 2000 22:133–153
10. Armitage CG (1999) Development of a classification system for periodontal diseases and conditions. Ann Periodontol 4:1–6
11. Hamp SE, Nyman S, Lindhe J (1975) Periodontal treatment of multirooted teeth. Results after 5 years. J Clin Periodontol 2:126–135
12. Hatit BY, Blum R, Severin C, Maquin M, Jabro MH (1996) The effects of a pulsed Nd:YAG laser in subgingival bacterial flora and on cementum: an *in vivo* study. J Clin Laser Med Surg 14(3):137–143
13. Löe H, Silness J (1963) Periodontal disease in pregnancy I. Prevalence and severity. Acta Odontol Scand 21:533–551

14. Silness J, Löe H (1964) Periodontal disease in pregnancy II. Correlation between oral hygiene and periodontal condition. Acta Odontol Scand 22:121–135
15. Renvert S, Wikström M, Dahlén G, Slots J, Egelberg J (1990) Effect of root debridement on the elimination of *Actinobacillus actinomycetemcomitans* and Bacteroides gingivalis form periodontal pocket. J Clin Periodontol 17:345–350
16. Möller AJR (1966) Microbiological examination of root canals and periapical tissues of human teeth. Odontol Tidskr 74(5, suppl 1):1–380
17. Slots J, Reynolds HS (1982) Long-wave UV light fluorescence for identification of black pigmented acteroides spp. J Clin Microbiol 16:1148–1151
18. Le Goff A, Bunetel L, Mouton C, Bonnaure-Mallet M (1997) Evaluation of root canal bacteria and their antimicrobial susceptibility in teeth with necrotic pulp. Oral Microbiol Immunol 12 (5):318–322
19. Alcoforado GAP, Mckay TL, Slots J (1987) Rapid method for detection of lactose fermentation oral microorganisms. Oral Microbiol Immunol 59:35–38
20. Slots J (1987) Detection of colonies of Bacteroides gingivalis by a rapid fluorescence assay for trypsin-like activity. Oral Microbiol Immunol 2:139–141
21. Morlock BJ, Pippin DJ, Cobb CM, Killoy WJ, Rapley JW (1992) 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 (7):637–641
22. Cobb CM, Mccawley TK, Killoy WJ (1992) A preliminary study on the effects of the Nd:YAG laser on root surface and subgingival microflora *in vivo*. J Periodontol 63(8):701–707
23. Radvar M, MacFarlane TW (1996) An evaluation of the Nd:YAG laser in periodontal pocket therapy. Br Dent J 180(2):57–62
24. Thomas D, Rapley J, Cobb C, Spencer PE, Killoy W (1994) Effects of the Nd:YAG laser and combined treatments on in vitro fibroblast attachment to root surfaces. J Clin Periodontol 21:38–44
25. Chan Y, Chien R (1994) Bactericidal action of Nd:YAG laser radiation in periodontal pockets. In: The 4th International Congress of Lasers in Dentistry, Singapore, pp 185–190
26. Pinero J (1998) Nd:YAG-assisted periodontal curettage to prevent bacteria before cardiovascular surgery. Dent Today 17(3):84–87
27. Lin PP, Rosen S, Beck FM, Matsue M, Horton JE (1992) A comparative effect of the Nd:YAG Laser with root planing on subgingival anaerobes in periodontal pockets. J Dent Res 71:299 (abstract 1547)
28. Neill ME, Mellonig JT (1997) Clinical efficacy of the Nd:YAG laser for combination periodontitis therapy. Pract Periodontics Aesthet Dent 9(6):1–5
29. Horton JE, Lin PP (1992) A comparison of the Nd:YAG laser used subgingivally with root planning [abstract 46]. 3rd International Congress Of Laser in Dentistry. Salt Lake City University of Utah, August 6–8:2
30. Severin C, Ragot-Roy B (1994) Pulsed Nd:YAG laser: bactericidal effect and dentin permeability. In: The 4th Int Congress Laser Dentistry, Bologna, pp 231–234
31. Tseng P, Gilkeson CF, Palmer J, Liew V (1991) The bactericidal effect of Nd:YAG laser *in vitro*. J Dent Res 70:650
32. Sbordone L, Ramaglia L, Gulletta E, Iacono V (1990) Recolonization of the subgingival microflora after scaling and root planing in human periodontitis. J Periodontol 61:579–584