

# The Short-term Effect of Diode Laser (980 nm) Treatment on Aggressive Periodontitis. Evaluation of Clinical and Microbiological Parameters

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**Purpose:** The purpose of the present study was to compare the short-term effect of scaling and root planing alone (SRP), diode laser (980 nm) treatment alone (LAS), and SRP combined with LAS (SRP+LAS) on clinical and microbial parameters in aggressive periodontitis (AgP).

**Materials and Methods:** Thirty AgP patients (14 men, 16 women) aged  $41.8 \pm 6.2$  years, 18 smokers, 12 non-smokers, participated in this study. Clinical assessments of plaque (PI), bleeding upon probing (BOP), probing pocket depth (PPD) and clinical attachment level (CAL) were made prior to treatment. Four plaque samples were randomly obtained from each individual, one in each quadrant. Following baseline (BL) clinical evaluation, each quadrant randomly received the following treatment modalities: SRP, SRP+LAS, LAS, CRL. A 980-nm diode laser (SmilePro980, Biolitec, Jena, Germany) was used for the laser treatment in a continuous focused mode and 2 W power setting. Subgingival plaque samples were collected from the same sites in each quadrant 2, 6, and 12 weeks after treatment. Clinical parameters were also recorded at the same time intervals. The level of *Porphyromonas gingivalis*, *Tannerella forsythia* (*Bacteroides forsythus*), *Actinobacillus actinomycetemcomitans*, *Treponema denticola*, and total bacterial load was evaluated using *ssrRNA* probes (IAI Pado Test 4.5, Institut für Angewandte Immunologie).

**Results:** Repeated measures analysis showed a significant time effect on bacterial counts, which were decreased following the three treatment modalities in all quadrants. They did not reach baseline levels 12 weeks after treatment. SRP+LAS showed lower bacterial levels than SRP or LAS at every time point after treatment. For *T. forsythia*, there was a significant time-by-treatment interaction effect, showing that each treatment had a different performance over time ( $F = 6.51$ ,  $p = 0.001$ ). Similar effects were observed for total bacterial load ( $F = 6.90$ ,  $p = 0.003$ ). Regarding *P. gingivalis* and *T. denticola*, time effects were significant ( $F = 6.84$ ,  $p = 0.009$ , and  $F = 12.45$ ,  $p < 0.001$ , resp). Treatment effects were significant for *P. gingivalis* ( $F = 7.96$ ,  $p = 0.003$ ). It is noteworthy that the SRP+LAS mean levels for all bacteria at the final follow-up point were never higher than the corresponding levels of the other treatments immediately after treatment (0.346 vs 0.369 for *T. forsythia*, 0.266 vs 0.548 for *P. gingivalis*, 0.155 vs 0.211 for *T. denticola*, and 3.580 vs 6.798 for total bacterial load. At the control sites, the bacterial counts showed no significant decrease.

**Conclusion:** Diode (980 nm) laser-assisted treatment with SRP showed a superior effect over SRP or LAS alone in both clinical and microbial parameters of AgP over a monitoring period of 12 weeks.

**Keywords:** aggressive periodontitis; microbiology; periodontal pathogens, scaling and root planing, laser treatment.

*J Oral Laser Applications* 2006; 6: 111-121.

Submitted for publication: 15.02.06; accepted for publication: 23.03.06.

Mechanical subgingival instrumentation consisting of scaling and root planing (SRP) is a widely used procedure for the treatment of inflammatory periodontal diseases; it is referred to as the gold-standard therapy. A number of studies based on site analysis have showed beneficial results in both clinical and microbial parameters.<sup>7</sup> The clinical benefits of SRP are derived from the disruption of the subgingival biofilm, reducing the bacterial load, therefore resulting in a delay in the repopulation of pathogenic microbiota.<sup>7,41</sup> The effect of SRP on selected bacterial species has been evaluated for shorter or longer periods of time.<sup>10,12,18,28,35</sup>

Biofilms associated with aggressive forms of periodontitis include *P.gingivalis*, *P. intermedia*, *T. forsythia*, *Campylobacter rectus*, and spirochetes.<sup>3,19-21,24,26</sup> Mechanical therapy alone may fail to eliminate these pathogenic bacteria because of their ability to invade within the periodontal tissues.<sup>31</sup>

Recently, the bactericidal and detoxification effects of lasers have been proposed as an alternative adjunctive technical modality to facilitate nonsurgical periodontal treatment.<sup>4,25,37</sup> In this context, good results have been obtained with 810-nm diode laser<sup>25</sup> and the pulsed Nd:YAG laser.<sup>15</sup>

The aim of the present study was to evaluate the short-term effect of 980-nm laser-assisted treatment on clinical and microbial parameters in AgP patients.

## MATERIALS AND METHODS

### Subjects

The study group consisted of 30 subjects with evidence of AgP, 14 men and 16 women, aged  $41.8 \pm 6.2$  years, 18 smokers and 12 nonsmokers. All subjects were treated by the same clinician (JK).

Patients were diagnosed as having AgP if they were under 35 years old and exhibited severe periodontal destruction, clinical attachment loss exceeding 5 mm at 2 to 3 sites in more than 14 permanent teeth (at least 3 of them were not first molars and incisors), and radiographic evidence of advanced alveolar bone loss.<sup>5</sup> Smoking was measured by self-report. All participants gave their consent to take part in the study.

### Periodontal Examination

Clinical examination included measurements of plaque,<sup>27</sup> bleeding upon probing,<sup>2</sup> suppuration,<sup>36</sup> all recorded as

dichotomous variables. Probing pocket depth (PPD) and clinical attachment level (CAL)<sup>14</sup> were measured to the nearest 1 mm using a Goldman/Fox Williams periodontal probe at 6 sites/tooth for all teeth present, excluding third molars. The number of teeth present was also recorded. One site with PPD > 5 mm was then randomly selected in each quadrant for microbial sampling (Table 1).

Clinical measurements and selection of sampling sites were performed one week before microbial sampling. Clinical parameters in sampling sites were re-examined immediately after microbial sampling, and these values were used in the analysis. All clinical measurements and microbial samplings were performed by one investigator (JK).

### Clinical Procedures

After baseline (BL) clinical and microbiological evaluation, subjects received oral hygiene instruction and supragingival scaling. Thereafter, each quadrant was randomly assigned to one of the following treatment modalities: scaling and root planing alone (SRP), diode (980 nm) laser treatment alone (LAS), and SRP combined with LAS (SRP+LAS). One quadrant was not treated, and served as control (CRL). SRP was performed mainly using Gracey curettes under local anesthesia. A 980-nm diode laser (SmilePro980, Biolitec, Jena, Germany) was used for the laser treatment in a continuous focused mode with 2 W power setting and a flexible glass optic fiber of 300  $\mu$ m diameter. The glass fiber end was calibrated to the pocket probing depth with a length approximately 1 mm less than the measured pocket depth. A shortening of 1 mm allowed irradiation of the pathogenic periodontal tissues without damage of the healthy attachment.

The flexible glass optic fiber was inserted into the bottom of the pocket parallel to the long axis of the root surface, aiming at the diseased soft tissue lining of the pocket (not toward the root surface), and was moved around the tooth. The fiber moved from the most apical point to the top of the pocket, making overlapping horizontal and vertical movements, maintaining contact with the soft tissue at all times. This procedure was repeated until the full circumference of the root was irradiated. Lasing was complete when signs of a new wound site (fresh bleeding) appeared. The total irradiation period for the whole procedure was approximately 30 s per tooth. This allowed laser-assisted soft-tissue curettage.

**Table 1 Demographic, behavioral and periodontal variables**

Characteristic	Aggressive periodontitis patients n = 30
Male/female	14/16
Age	41.8 ± 6.2 years
Smokers	18 (31.1 ± 8.7 cig/day)
Number of teeth	25.5 ± 1.9
Mean PPD in mm/sampling sites	6.3 ± 0.3
Mean CAL in mm/sampling sites	6.9 ± 0.1
Plaque†	59.1 ± 3.1
Bleeding on probing†	86.9 ± 9.8
† Mean % positive sites	

### Bacterial Sampling and Analysis

Four plaque samples were randomly obtained from each individual, one site with PPD > 5 mm in each quadrant. One paper point was inserted into each pre-selected periodontal pocket for 10 s and used for DNA probe analysis. Subgingival plaque samples were collected from the same sites in each quadrant at 2 (2wk), 6 (6wk), and 12 weeks (12wk) after treatment. The level of *P. gingivalis*, *T. forsythia*, *A. actinomycetemcomitans*, *T. denticola*, and total bacterial load were evaluated using the IAI Pado Test 4.5, (Institut für Angewandte Immunologie, Zuchwil, Switzerland). Samples were processed by standard procedures and hybridized with <sup>32</sup>P-labeled specific probes for the small subunit ribosomal RNAs (ssrRNAs) of *P. gingivalis*, *T. forsythia*, *A. actinomycetemcomitans*, *T. denticola* and a universal bacterial probe.<sup>11</sup> The values for each bacterial species were computed by comparison with a homologous standard of each bacterium. The total bacterial count was determined using the universal probe. The results were translated into millions of bacteria by arbitrarily deciding that one bacterium was equivalent to 10<sup>4</sup> copies of ssrRNA.

### Statistical Analysis

Friedman tests were used for testing mean differences between treatment groups at baseline for all bacterial species. Since each individual served as a block for testing both treatment and time effects (post therapy), the study design resembled a split-split-plot one. Univariate

repeated-measures ANOVA with two within-individual factors was used. Modified F-tests with Greenhouse-Geisser correction and Wilk's Lambda multivariate test were used to seek the main effects of the different treatment modalities (SRP, SRP+LAS, LAS, CRL) on the level of the bacterial species and clinical parameters CAL and PPD.

These tests along with Wilcoxon tests and paired t-tests were also used to determine the effect of treatment modalities (SRP, SRP+LAS, LAS, CRL) over time (BL, 2wk, 6wk, 12wk post-therapy).

### RESULTS

Demographic data are shown in Table 1. Age was not different between genders ( $t = 0.85$ ,  $p = 0.411$ ), nor was PPD ( $t = 1.25$ ,  $p = 0.235$ ). However, CAL was greater for males ( $t = 3.51$ ,  $p = 0.004$ ). A series of Friedman tests showed that there were no statistical differences among bacterial species at BL, namely *A. actinomycetemcomitans* ( $p = 0.954$ ), *T. forsythia* ( $p = 0.056$ ), *P. gingivalis* ( $p = 0.695$ ), *T. denticola* ( $p = 0.567$ ), as well as total bacterial load ( $p = 0.313$ ).

Table 2 presents mean concentrations of bacterial species for each treatment modality after therapy. The improvement following treatment is evident for all bacterial species apart from *A. actinomycetemcomitans*, possibly because of the low values that were detected. SRP+LAS was the most effective, since the level of the bacterial species was decreased to undetectable levels in the majority of cases. In general, the formal analysis showed significant post-therapy (time) effects on bacte-



**Table 2 Means per treatment group for all bacterial species post-therapy (standard deviations in parenthesis)**

Bacterial species	Treatment	Time			
		Baseline	2 wk	6 wk	12 wk
A. actinomycetemcomitans	SRP	0.023† (0.044)	0.008 (0.026)	0.014 (0.005)	0.022 (0.077)
	SRP+LAS	0.025 (0.069)	0.000 (0.0)	0.009 (0.012)	0.016 (0.033)
	LAS	0.093 (0.278)	0.007 (0.026)	0.021 (0.041)	0.040 (0.094)
	CRL	0.063 (0.175)	0.065 (0.190)	0.036 (0.022)	0.040 (0.094)
T. forsythia	SRP	1.852 (1.636)	0.369 (0.685)	0.378 (0.657)	0.553 (0.662)
	SRP+LAS	2.134 (1.471)	0.153 (0.387)	0.148 (0.313)	0.178 (0.345)
	LAS	2.002 (1.803)	0.360 (0.499)	0.315 (0.517)	0.378 (0.596)
	CRL	1.207 (1.359)	1.436 (1.425)	1.006 (0.599)	0.905 (0.640)
P. gingivalis	SRP	2.695 (2.987)	0.548 (1.115)	0.598 (1.108)	0.672 (1.166)
	SRP+LAS	2.740 (2.792)	0.073 (0.279)	0.063 (0.277)	0.094 (0.296)
	LAS	2.629 (2.357)	0.288 (0.433)	0.297 (0.526)	0.311 (0.584)
	CRL	2.461 (3.199)	2.370 (3.042)	2.008 (1.967)	2.128 (1.792)
T. denticola	SRP	0.483 (0.423)	0.211 (0.277)	0.289 (0.312)	0.313 (0.452)
	SRP+LAS	0.900 (0.525)	0.126 (0.346)	0.128 (0.218)	0.098 (0.223)
	LAS	0.678 (0.573)	0.201 (0.247)	0.242 (0.208)	0.235 (0.333)
	CRL	0.828 (0.952)	0.399 (0.349)	0.489 (0.434)	0.560 (0.519)
Total bacterial load	SRP	36.706 (27.513)	6.798 (6.247)	8.642 (6.993)	10.337 (9.710)
	SRP+LAS	35.835 (17.119)	3.034 (1.881)	3.121 (1.622)	2.763 (1.910)
	LAS	35.236 (28.473)	5.989 (4.781)	6.232 (4.631)	6.704 (4.568)
	CRL	32.734 (21.932)	27.511 (19.696)	27.349 (15.976)	27.277 (16.774)

† × 10<sup>6</sup>

rial counts, which decreased following the three treatment modalities in all quadrants. They did not reach baseline levels 12 weeks after therapy.

Table 3 shows the p values for treatment effects, time (post-therapy) effects and treatment-by-time in-

teractions. A. actinomycetemcomitans did not show any treatment-by-time interaction effects ( $F = 0.872$ ,  $p = 0.435$ ), time effects ( $F = 0.362$ ,  $p = 0.617$ ), or treatment effects ( $F = 1.73$ ,  $p = 0.215$ ).

**Table 3 Results\* from testing main and interaction effects of time and treatment modalities on bacterial species**

	A. actinomyce temcomitans	T. forsythia	P. gingivalis	T. denticola	Total bacterial load
Time x treatment modality	p=0.435 F=0.872	<b>p=0.001</b> F=6.51	p=0.101 F=2.02	p=0.099 F=1.98	<b>p=0.003</b> F=6.90
Time	p=0.617 F= 0.362		<b>p=0.009</b> F= 6.84	<b>p&lt;0.001</b> F= 12.45	
Treatment modality	p=0.215 F= 1.73		<b>p=0.003</b> F= 7.96	p=0.077 F= 3.16	

\*Univariate repeated measures ANOVA tests with Greenhouse-Geisser correction for the degrees of freedom.  
**Bold face denotes the statistically significant results.**

**Table 4 Mean differences ( $\sigma$ ) between BL and 2wk ( $\sigma$  1,2) and between 2wk and 12wk ( $\sigma$  2,4) for all treatment groups for T. forsythia and total bacterial load. Also shown differences of ( $\sigma$  1,2 and  $\sigma$  2,4) between SRP+LAS and the other treatment groups (standard deviations in parentheses)**

		T. forsythia			
		CRL	LAS	SRP	SRP+LAS
$\sigma$ 1,2		-0.229 (1.015)	1.642 (1.565)	1.483 (1.834)	1.981 (1.585)
$\sigma$ 2,4		-0.292 (1.832)	-0.477 (1.023)	-0.180 (0.629)	-0.182 (0.337)
<b>Difference with SRP+LAS</b>					
	<b>s 1,2</b>		<b>p</b>	<b><math>\sigma</math> 2,4</b>	<b>p</b>
SRP		-0.498 (1.318)	0.394	-0.031 (0.679)	0.600
LAS		-0.339 (1.462)	0.570	-0.329 (0.941)	0.308
CRL		-2.210 (1.387)	0.001	-0.212 (1.938)	0.551
		Total bacterial load			
		CRL	LAS	SRP	SRP+LAS
$\sigma$ 1,2		5.223 (7.350)	29.246 (25.826)	29.907 (22.829)	32.801 (15.906)
$\sigma$ 2,4		-0.782 (5.403)	-1.503 (3.487)	-3.374 (3.288)	-0.547 (1.306)
<b>Difference with SRP+LAS</b>					
	<b><math>\sigma</math> 1,2</b>		<b>P</b>	<b><math>\sigma</math> 2,4</b>	<b>p</b>
SRP		-2.894 (13.823)	0.427	-2.827 (3.165)	0.005
LAS		-3.555 (26.302)	0.281	-0.956 (2.823)	0.256
CRL		-27.578 (18.168)	0.001	-0.235 (6.017)	0.496

p values refer to Wilcoxon tests

T. forsythia levels presented a significant time-by-treatment (post-treatment periods) interaction effect, showing that each treatment modality had a different performance on time (F = 6.51, p = 0.001). The interaction seems to be due to the control group. Apart from the control group, the three treatment modalities had similar performances. SRP+LAS had the best effect in all post-treatment periods. This is also shown in

Table 4, where the difference between baseline and 2 wk and the difference between 2 wk and 12 wk are shown for all treatments, along with the differences of these values between the SRP+LAS with the other groups. SRP+LAS presented the greatest decrease in bacterial counts with respect to baseline (1.981), and the differences with the other groups seem negligible except for the control group. P. gingivalis levels pre-

**Table 5 Mean differences for *P. gingivalis* and *T. denticola* between measurements on time BL- 2wk ( $\sigma$  1,2), BL-12wk ( $\sigma$ 1,4), 2wk-12wk ( $\sigma$  2,4) and between treatment groups along with statistical significance (standard deviations in parentheses)**

		<b><i>P. gingivalis</i></b>			
<b>Differences with SRP+LAS</b>	<b>CRL</b>	<b>LAS</b>	<b>SRP</b>		
	Mean	1.496 (2.689)	0.175 (1.322)	0.493 (1.537)	
	p	<0.001	0.313	0.017	
<b>Differences between times (<math>\sigma</math>)</b>	<b><math>\sigma</math> 1,2</b>	<b><math>\sigma</math> 1,4</b>	<b><math>\sigma</math> 2,4</b>		
	Mean	1.812 (2.917)	1.586 (2.941)	-0.225 (1.768)	
	p	<0.001	<0.001	0.328	
		<b><i>T. denticola</i></b>			
<b>Differences with SRP+LAS</b>	<b>CRL</b>	<b>LAS</b>	<b>SRP</b>		
	Mean	0.306 (0.694)	0.086 (0.496)	0.074 (0.637)	
	p	<0.001	0.189	0.371	
<b>Differences between times (<math>\sigma</math>)</b>	<b><math>\sigma</math> 1,2</b>	<b><math>\sigma</math> 1,4</b>	<b><math>\sigma</math> 2,4</b>		
	Mean	0.488 (0.618)	0.232 (0.824)	-0.256 (0.534)	
	p	<0.001	0.033	<0.001	

p values refer to Wilcoxon tests

sented a significant time effect ( $F = 6.84$ ,  $p = 0.009$ ) and a significant treatment effect ( $F = 7.96$ ,  $p = 0.003$ ), but no time-by-treatment interaction ( $F = 2.02$ ,  $p = 0.101$ ) (Table 3). Specifically, as can be seen from Table 5, the differences between the BL and the 2wk or the 12wk are both statistically significant ( $p < 0.001$ ), showing a general improvement after therapy. This improvement seems to be greater for the SRP+LAS group (mean difference from the control group = 1.496,  $p < 0.001$ ) although it is not statistically significant from the other groups (0.175,  $p = 0.313$ , and 0.493,  $p = 0.017$  from the LAS and SRP groups, respectively). *T. denticola* presented a significant time effect ( $F = 12.45$ ,  $p < 0.001$ ) but no treatment group or group-by-time interaction effects ( $F = 3.16$ ,  $p = 0.077$  and  $F = 1.89$ ,  $p = 0.250$ , respectively) (Table 3). Table 5 shows the improvement in *T. denticola* mean measurements between BL and 2wk. A significant deterioration, however, is noticed after 2 weeks (mean difference with 12wk = -0.256,  $p < 0.001$ ). Total bacterial load presented a statistically significant treatment-group-by-time interaction ( $F = 6.90$ ,  $p = 0.003$ ) (Table

3). Again as in the *T. forsythia* case, the interaction seems to be due to the control group, which had an almost unaltered bacterial count, as expected, throughout the monitoring period. As only *T. forsythia* and total bacterial load showed statistically significant time-by-treatment interactions, these two are presented in Table 4. Table 4 shows the great improvement over time after therapy, at least for the SRP+LAS group (mean difference between baseline and 2 wk = 32.801). The differences of these improvements with the other groups were not statistically significant, apart from the difference between 2 wk and 12 wk and between the SRP and SRP+LAS groups (-2.827,  $p = 0.004$ ).

For the better exploitation of the information given by the levels of bacterial species, it was decided to consider thresholds to transform the continuous into polytomous data. The thresholds suggested for bacterial isolation were equal to  $\times 10^4$  and  $\times 10^2$ . There were very few data in the interval between  $\times 10^4$  and  $\times 10^2$ . Therefore, the bacterial levels were dichotomized using one threshold at  $\times 10^2$ . Table 6 shows the percentages

Table 6 Percentages of presence of bacteria per time point and treatment modality					
<u>A. actinomycetemcomitans</u>					
Time	BL	2wk	6wk	12wk	X <sup>2</sup> (p-value)
	33.3%	17.2%	12.2%	12.4%	4.502 (0.212)
Treatment	CRL	LAS	SRP	SRP+LAS	
	37.3%	19.6%	29.3%	8.8%	14.473 (0.002)
<u>T. forsythia</u>					
Time	BL	2wk	6wk	12wk	X <sup>2</sup> (p-value)
	95%	61.7%	77.6%	78%	19.476 (<0.001)
Treatment	CRL	LAS	SRP	SRP+LAS	
	93.3%	76.3%	80%	62.1%	17.074 (0.001)
<u>P. gingivalis</u>					
Time	BL	2wk	6wk	12wk	X <sup>2</sup> (p-value)
	83.3%	45%	77.8%	65%	19.680 (<0.001)
Treatment	CRL	LAS	SRP	SRP+LAS	
	88.3%	66.7%	61.7%	44.1%	26.175 (<0.001)
<u>T. denticola</u>					
Time	BL	2wk	6wk	12wk	X <sup>2</sup> (p-value)
	91.7%	63.3%	69.5%	83.3%	16.846 (0.001)
Treatment	CRL	LAS	SRP	SRP+LAS	
	93.3%	74.6%	83.3%	56.7%	24.591 (<0.001)

Table 7 Odds ratios for the presence of bacteria and their significances from 1 for each treatment as compared with SRP+LAS			
		Odds ratio with SRP+LAS	p-value
A. actinomycetemcomitans	CRL	6.184	0.001
	LAS	2.542	0.106
	SRP	4.312	0.008
T. forsythia	CRL	8.556	<0.001
	LAS	1.964	0.098
	SRP	2.444	0.034
P. gingivalis	CRL	9.610	<0.001
	LAS	2.538	0.014
	SRP	2.042	0.056
T. denticola	CRL	10.706	<0.001
	LAS	2.243	0.042
	SRP	3.824	0.002

**Table 8 Comparison of differences ( $\sigma$ ) in PPD and CAL from baseline to 12 wk post treatment and between SRP+LAS and the other treatment modalities (standard deviations in parenthesis)**

	$\sigma$ PPD in mm BL-12 wk	$\sigma$ CAL in mm BL-12 wk	$\sigma$ PPD in mm	$\sigma$ CAL in mm
SRP+LAS	2.80 (0.77) p*=0.001	2.14 (1.60) p*=0.001		
SRP	2.34 (0.82) p=0.001	1.87 (0.92) p=0.001	0.47 (0.83) p**=0.053	0.27 (1.22) p**=0.429
LAS	2.00 (0.38) p<0.001	1.94 (0.96) p=0.001	0.80 (0.77) p=0.006	0.20 (1.37) p=0.713
CRL	0.13 (0.35) p=0.157	0.27 (0.59) p=0.102	2.67 (0.82) p=0.001	1.87 (1.81) p=0.003
* : Wilcoxon tests				
** : Univariate repeated measures ANOVA test with Greenhouse-Geisser correction for the degrees of freedom				

of *A. actinomycetemcomitans*, *T. forsythia*, *P. gingivalis* and *T. denticola* presence (threshold at  $\times 10^2$ ) per time point post-treatment and per treatment modality along with  $\chi^2$  values. It is observed that these percentages of presence are in general different for different time points and between treatments. The lowest percentage of presence of all bacteria is noticed at 2 weeks after treatment. SRP+LAS showed the lowest bacteria percentages among all treatments modalities ( $p < 0.001$ ). Since there is a particular interest in the differences between treatments modalities, Table 7 presents the estimated odds ratios for the presence of bacteria between each treatment modality and SRP+LAS along with their statistical significances. The results show that LAS has odds of presence of *A. actinomycetemcomitans*, *T. forsythia* and *T. denticola* closer to SRP+LAS than all other treatments. Further, for *A. actinomycetemcomitans* and *T. forsythia*, these odds for LAS and SRP+LAS are statistically the same. SRP and SRP+LAS have the same odds for *P. gingivalis*; this result is of limited statistical significance.

PPD and CAL presented a statistically significant time post-therapy by treatment interaction ( $F = 54.60$ ,  $p < 0.001$  and  $F = 11.72$ ,  $p < 0.001$  for PPD and CAL, respectively) (data not shown in Tables). Table 8 shows the differences between the BL and 12wk in PPD and CAL for all treatment groups and how much these differences differ between groups. All treatment modalities showed significant improvement in PPD and CAL values from BL to 12 wk post-therapy. It is also seen that SRP+LAS was statistically significantly more effective

in reducing PPD and CAL values than were CRL and LAS. Figure 1 displays the differences between the BL and 12wk in BOP for all treatment groups. A substantial decrease in BOP was observed in all treatment groups with more positive outcome in SRP+LAS at the end of the observation period.

## DISCUSSION

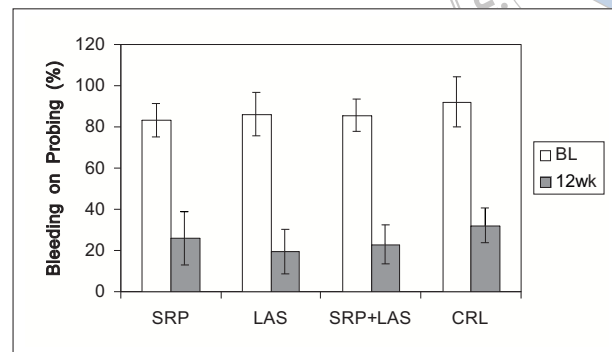
### Microbial Evaluation

The present clinical study evaluated the effectiveness of a diode laser (980 nm) treatment on clinical and microbiological parameters of patients with aggressive periodontitis.

This is the first study to our knowledge that has confirmed the in vivo antibacterial effectiveness of a 980-nm diode laser in periodontology. Moritz et al<sup>25</sup> reported considerable bacterial elimination from periodontal pockets using irradiation with an 810-nm diode laser with 2.5 W power settings in pulsed mode (50 Hz, pulse duration 10 ms) following scaling as compared to scaling alone.

Scaling and root planing is one of the most commonly utilized procedures for the treatment of periodontal diseases and has been used as the gold-standard treatment. Mechanical treatment alone has been shown to be clinically effective.<sup>6</sup> Numerous studies have reported beneficial results from this treatment in both clinical and microbial parameters. The clinical





**Fig 1** BOP % values at BL and 12 wk in all treatment groups

benefits are derived from the removal of subgingival plaque and disruption of subgingival biofilm leading to a decrease of bacterial counts.

In the present study, SRP alone was capable of appreciably decreasing the counts of the bacterial species tested, namely, *P. gingivalis*, *T. forsythia*, *A. actinomycetemcomitans*, *T. denticola* as well as total bacterial load. These data confirm the favorable effect of SRP in decreasing the levels of *P. gingivalis* and *T. denticola* that has previously been reported.<sup>10,18,34,40</sup>

The laser-assisted treatment (SRP+LAS) showed lower bacterial levels as compared to SRP or LAS at every time point after treatment.

As shown in the present study, SRP+LAS was the most effective treatment modality, keeping the levels of all bacterial species at very low even 12 weeks after therapy. The most favorable bacterial reduction was achieved 2 weeks post-therapy for all the bacterial species tested, although all species were still significantly reduced or eliminated at 12 weeks when compared to pretreatment levels. This favorable effect might be due to the ability of laser irradiation to eliminate bacteria in the dentinal tubules where they can act as a “reservoir” for recolonization and re-infection of the pocket.<sup>1,13,16</sup>

While SRP is the most commonly used periodontal therapy for the cause-related phase of treatment, there are limitations, including the inability to adequately instrument deep periodontal pockets and furcations as well as remove microorganisms within the tissues lining the periodontal pocket. Darby et al<sup>10</sup> have shown that scaling and root planing resulted in clinical improvement and significant reduction in the levels of *P. intermedia*, *T. forsythia* and *T. denticola*. Renvert et al<sup>28</sup> demonstrated similar results. However, *A. actinomycetemcomitans* still remained in a high proportion of sites after therapy, probably due to its ability to invade periodontal tissues. It is known that periodontal

pathogens are capable of invading periodontal tissues. *A. actinomycetemcomitans* was found in the connective tissue of active as compared to nonactive sites.<sup>31</sup> In addition, *P. gingivalis* can adhere and enter oral epithelial cells.<sup>32</sup> Our results indicated that SRP alone was not able to eliminate *A. actinomycetemcomitans*, while laser-assisted curettage succeeded in eliminating this bacterium after the combined therapy. Laser-assisted treatment had an excellent effect on the counts of *T. forsythia*, another periodontal pathogen which, according to clinical studies, is resistant to elimination by scaling and root planing.<sup>10,18,38</sup>

Lasers have been introduced as an adjunctive tool to mechanical therapy. They have the potential of a bactericidal and detoxification effect. Gutknecht et al<sup>16</sup> showed that a 980-nm diode laser can eliminate bacteria that have immigrated deep into the dentin, and were thus able to increase the success rate in endodontic therapy, while the 980-nm diode laser used as an adjunct to SRP facilitated bacterial elimination from periodontal pockets at a higher level than SRP alone.<sup>25</sup>

It is noteworthy that the SRP+LAS mean levels for all bacteria at the final follow-up point (12wk) were never higher than the corresponding levels of the other treatments 2 weeks after therapy. These findings are consistent with results from previous studies, which have shown that short-term reduction in the level of bacteria occurred during the first 3 months post-therapy,<sup>9,34</sup> and that bacterial recolonization occurred after 70 days to 3 months.<sup>23,33</sup>

### Clinical Evaluation

A considerable body of evidence indicates that mechanical instrumentation is effective in suppressing periodontal pathogens and promoting clinical improvement. It is the first necessary step in treatment planning for



all forms of periodontal diseases. Its limitations have already been mentioned. The need of a more powerful periodontal therapy for aggressive periodontitis, which represents the most severe and rapidly destructive form of periodontal diseases, is highly desirable. Lasers have been introduced as an adjunctive tool to mechanical instrumentation as they have been shown to have a bactericidal and detoxification effect and better clinical parameters than SRP alone.<sup>25</sup> The present study suggests that diode-laser-assisted periodontal treatment with SRP seems to have a superior effect to SRP and LAS alone on the microbiological variables of AgP and a better clinical outcome than LAS alone over the 12-week monitoring period.

Collectively, laser-assisted treatment when combined with subgingival debridement showed a substantial clinical improvement due to the favorable alterations detected in the subgingival microflora, thereby indicating that it is effective in the cause-related treatment of AgP where anaerobic bacteria are predominant. Therefore, this adjunctive therapy should not replace mechanical instrumentation but rather complement it.

Regarding clinical use of the diode laser for periodontal treatment, Coluzzi<sup>8</sup> recommended laser soft-tissue curettage at 0.4 W in continuous wave mode before mechanical debridement of root surfaces, and 0.6 W afterwards for hemostasis and bacterial reduction. Gutknecht et al<sup>17</sup> suggested the use of a diode laser at 2 W in continuous wave mode for curettage before mechanical debridement both to reduce the risk of bacteremia and facilitate mechanical debridement. Since the 980-nm diode laser does not interact with the hard dental tissues, it is an excellent soft-tissue laser instrument.<sup>30</sup> Complete epithelial removal has been shown after a 980-nm diode laser application compared to the mechanical debridement with curettes.<sup>29</sup> Recent studies have revealed that root surface irregularities were more pronounced after irradiation with an Er:YAG laser than with a diode laser.<sup>39</sup>

As the 1989 and 1996 reviews in "World Workshops on Periodontology"<sup>42,43</sup> indicated, there is controversy about the necessity for gingival curettage in the mechanical treatment of periodontitis. However, the presence of invading bacteria in deeper parts of dentinal tubules suggests that a purely mechanical therapy of SRP is unable to reach and eliminate these bacteria, especially in aggressive cases of periodontitis. Moreover, such bacterial invasion of root structure may represent a reservoir of periodontopathic bacteria for recolonization and re-infection.<sup>1,13</sup> There are studies indicating that diode laser irradiation can destroy bacteria in the dentinal tubules and can penetrate deeper than chemicals.<sup>16,22</sup>

Considering the various advantages of laser irradiation, its use in combination with mechanical instrumentation has the potential to improve the environment of periodontal pockets. Furthermore, given the evidence of bacterial invasion within the soft tissues of periodontal pockets, not only debridement of the root surface but also the removal of the pocket epithelium and granulation tissue (which can be accomplished by laser irradiation) could be important factors in promoting attachment of the connective tissue to the root surface.

Therefore, more clinical studies are needed to elucidate the effect of laser in comparison with mechanical instrumentation.

## ACKNOWLEDGEMENTS

We gratefully acknowledge the support of BIOLITEC (Jena, Germany) for providing us with their SmilePro980 laser equipment. The authors would also like to thank Prof. Pierre Baehni for the helpful discussion throughout the study.

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