

# Laser Supported Reduction of Specific Microorganisms in the Periodontal Pockets with the Aid of an Nd:YAG Laser

## An in vivo Study

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### \_Abstract

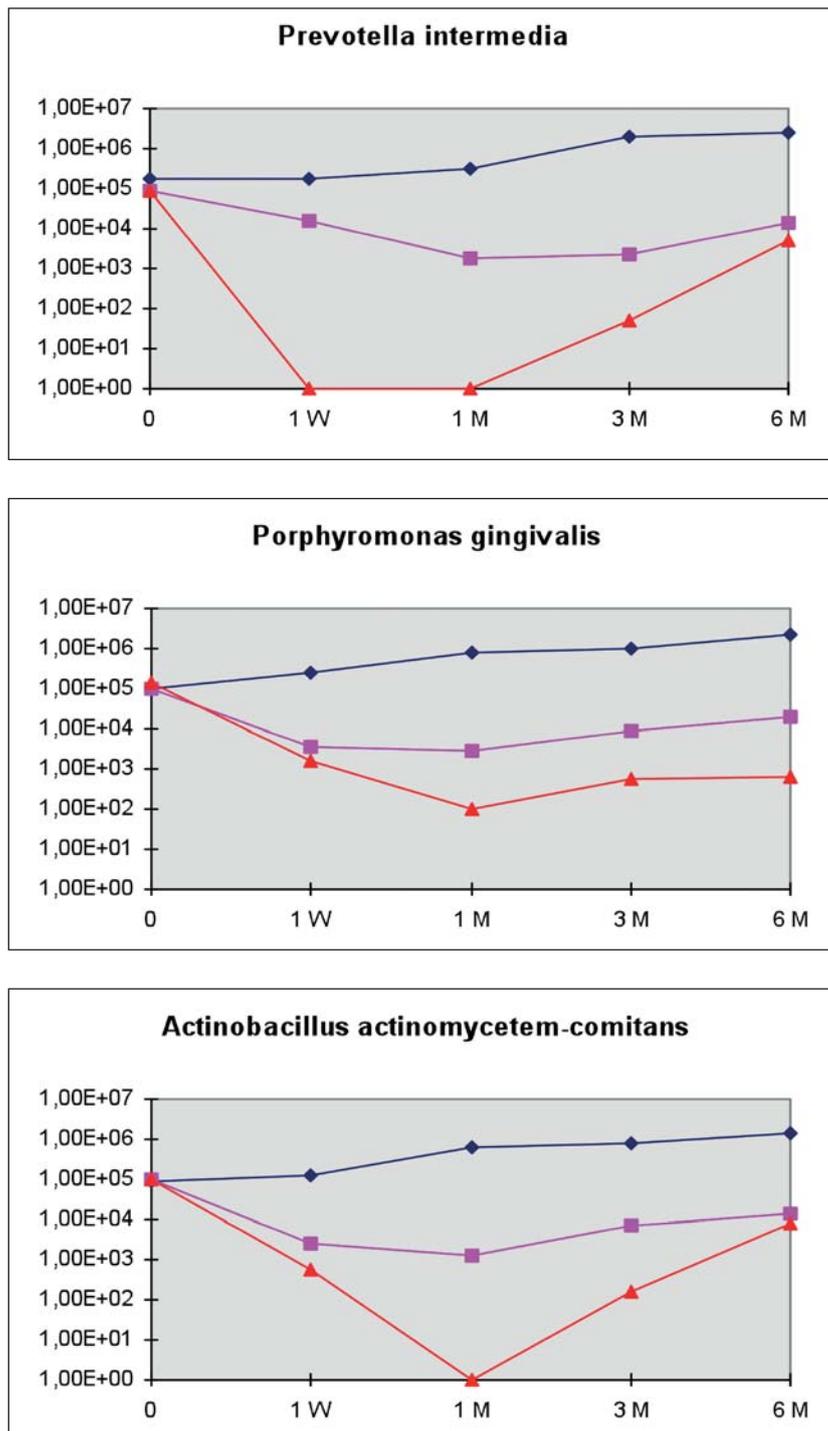
*Objective:* We investigated the application of the Nd:YAG laser as an adjuvant possibility of treating periodontitis compared to conventional treatment on its own. *Summary Background Data:* To free the subgingival surroundings from periodontopathogenic germs—an essential aim in the clinic for periodontology—the use of laser seems to be particularly suitable owing to its germ-reducing effect known especially from endodontics. Good results were achieved with the pulsed Nd:YAG laser. *Methods:* The clinical study we performed comprised twenty patients; the bacterial count serving as a microbiological and the probing depth, ie the haemorrhagic tendency, as the clinical examination parameter. Quantitative proof of three periodontopathogenic germs (actinobacillus actinomycetem-comitans, prevotella intermedia and porphyromonas gingivalis) was produced in each case with both a digoxigenin-marked 16S-rRNA probe and a genomic DNA probe. To determine the clinical parameter, a CPC 11 periodontal probe was applied. Three quadrants per patient and, in each quadrant, one periodontium in need of treatment were fixed as relevant to the study. In each case, one periodontium was left untreated as a control, a second one was treated with a conventional subgingival curettage only, whereas the third was treated both conventionally and with laser. All examination parameters were determined in each quadrant before and one, three and six months after the particular treatment. In addition, we determined a bacterial count one week after treatment. *Results:* We observed that the average values of the absolute bacterial count in the quadrant that had been treated with the adjuvant laser always lay below the values that corresponded to the quadrants that had only been treated conventionally. There were statistically significant differences in favour of the laser method especially during the measurements one week and one month after treatment. Also the clinical parameters, like the probing depth and the haemorrhagic tendency, had been positively influenced by the adju-

vant laser therapy. *Conclusion:* The effectiveness of a periodontitis treatment supported by laser was able to be demonstrated. With regard to the desired elimination of germs, the application of the Nd:YAG laser proves to be a sensible and complementary therapeutic measure.

### \_Introduction

Inflammatory forms of periodontopathy are the most common disease of the periodontium and can lead to the loss of the affected tooth if left untreated. Inflammations of the periodontium are based on tissue reactions that are caused by localised supra- and subgingival microbial plaque. Regarding the composition of pathogenous plaque, the specific plaque hypothesis<sup>1</sup> has become more and more established in the last few years according to which only a few—20 at most—of over 300 different bacterial species which have so far been able to be isolated from plaque samples are associated with the destruction of periodontal tissue. Especially the black pigmented, gram-negative anaerobes porphyromonas gingivalis (P.g.) and prevotella intermedia (P.i.), and the facultative anaerobe actinobacillus actinomycetem-comitans (A.a.) seem to be the main pathogenic agents in progressing periodontitis in man. These bacteria have been detected again and again in a high bacterial count in the destructive forms of periodontitis, and they are considered to be the indicator organisms of this disease.<sup>2,3</sup>

An important aim in the causal treatment of periodontitis consists therefore in the radical elimination of the pathogenic germs and preventing a subsequent recolonisation of the periodontal pockets. Seeming that the instrumental curettage still remains to be the indispensable method chosen, the application of laser is gaining more and more in importance as an adjuvant possibility of therapy. There have been repeated reports on the successful application of both the CO<sub>2</sub> and Nd:YAG lasers in the treatment of periodontitis—the clinical results having served as the



**Diagram 1** The arithmetic means of the absolute bacterial count measured with the digoxigenin-marked 16S-rRNA probe in logarithmic scales before (0), ie one week (1 W), 1, 3 and 6 months (1 M, 3 M, 6 M) after the particular therapy. Conventional curettage (■), additional Nd:YAG laser treatment (▲), no treatment (◆).

main criteria in most cases.<sup>14,15</sup> The aim of this in vivo study consisted in investigating the bactericidal effectiveness of the Nd:YAG laser on the pigmented germs P.i. and P.g., but also on the species A.a. which, to a certain extent, is considered to be a problematic germ. Our observations here were based on very sensitive and, at the same time, specific methods of germ detection. The decision in favour of the Nd:YAG laser was based on the by far greater experience with the same in endodontics and in the assumption of being able to apply the results especially regarding the elimination or reduction of germs.<sup>16, 17, 18</sup>

## Materials and Methods

In this study, twenty adult patients were taken in who showed periodontally affected teeth in at least three quadrants (one tooth at least with a pocket depth of 4-6 mm per quadrant). All such cases were excluded in which periodontal treatment and/or treatment with antibiotics had taken place not longer than three months earlier; furthermore, patients with contagious diseases, pregnant women and nursing mothers were also excluded.

For the subgingival curettage, conventional Gracey curettes were used and an Nd:YAG laser was applied in the laser treatment for which the following adjustments had been chosen: frequency: 20 Hz; energy: 100 mJ; average output: 2 W; pulse length: 100  $\mu$ s. With the aid of a 320  $\mu$ m thick quartz fibre, the bottom of the pocket was irradiated circularly for 40 seconds parallel to the surface of the root (energy density directly on the emission surface of the fibre: 124 J/cm<sup>2</sup>). This happened three times at an interval of one week each.

The detection of germs took place with both a digoxigenin-marked 16S-rRNA probe and a genomic DNA probe. Statements on the absolute cellular count cannot be made with this method, as the DNA probes are directed against 16S-rRNA, resulting in a variability of the RNA molecular count per bacterial cell dependent on its "physiological condition". The derivation of the cellular count from the 16S-rRNA detection is thus a semi-quantitative value.<sup>4</sup>

There is no significant deviation between the quantification with DNA probes and the culture technique. In a comparative investigation performed by CONRADS and BRAUNER (1993)<sup>5</sup>, it was discovered that there was a high conformity between the culture technique and the DNA probe detection if the samples were rapidly preserved at -20 °C and the preparation of the nucleic acid took place free of loss. In our study, we investigated the three species A.a., P.i. and P.g.

With each patient, three quadrants and, in each quadrant, a periodontium in need of treatment were fixed as relevant to the study. Always one periodontium was left untreated over the entire study as a control, another periodontium was only treated once with the conventional subgingival curettage, whereas the third periodontium—in addition to the conventional initial therapy—received laser treatment, consisting of three visits at one week intervals.

The microbiological parameters (determination the bacterial count for A.a., P.g. and P.i.) were determined both one week after treatment, and one, three and six months after having concluded each treatment. Determining the clinical parameters (haemorrhagic tendency and pocket depth) took place at the same time as determining the bacterial count through careful instrumental probing.

Parting from the rough data thus obtained, we first proved for every investigation parameter on the basis

of a monofactorial variance analysis that the gathered data in the untreated quadrant (control value) showed no statistically significant differences over the entire study period.

Afterwards, the post-therapeutically established data in each treated quadrant was checked with the initial data with the help of a paired t test for statistically significant differences. Finally, the data gathered in the quadrants that had been additionally treated with laser was compared with the data of the conventionally treated quadrant as part of an unpaired t test.

## Results

### Microbiological Parameters

#### 1. *Prevotella intermedia*:

The investigations showed that the bacterial count after the adjuvant laser treatment decreased significantly and remained at a significantly low level over the entire observation period. After conventional treatment alone, both analyses first also showed a significant decrease in the bacterial count compared to the initial stadium. However, as from the third month in the genomic DNA analysis and as from the sixth month in the 16S-RNA analysis, the differences in the bacterial count were no longer statistically significant compared to the initial stadium. The differentiated statistical comparison between the conventional and the adjuvant laser method results in statistically significant differences in the bacterial count after one week, one month and three months in favour of the laser method (cf. Diagram 1a).

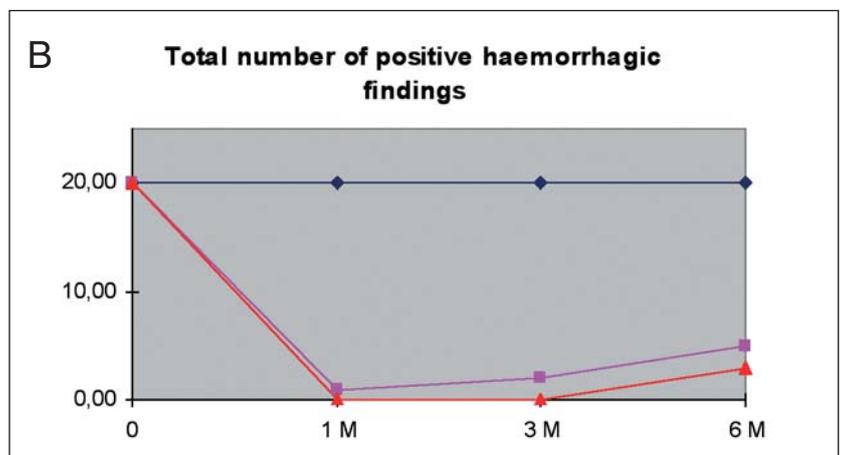
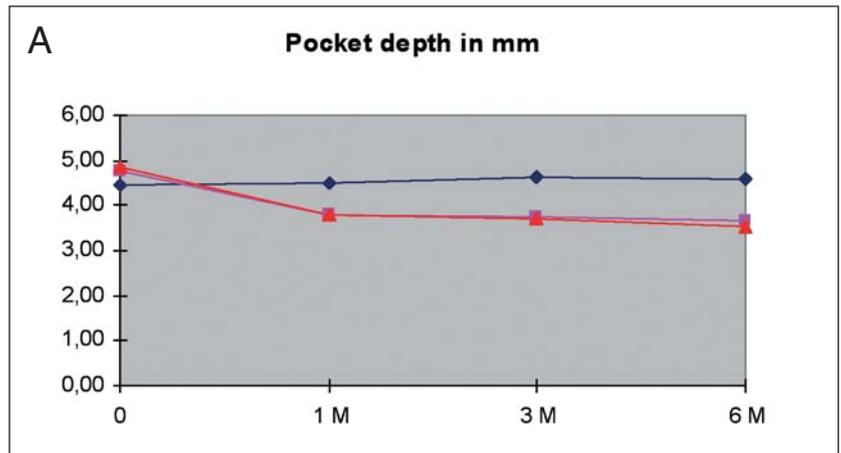
#### 2. *Porphyromonas gingivalis*:

Both in the DNA and RNA analyses, a significant decrease in the bacterial count after both methods of treatment were able to be observed over the entire study period. The simple comparison of the average bacterial count showed, especially at first, a clearly stronger decrease after the adjuvant laser therapy than after the conventional method (see Diagram 1b). The statistical evaluation, however, showed no significant difference in the DNA analysis, whereas the measurements in the RNA analysis after one month and after six months were able to prove a significantly stronger decrease of the detectable bacterial count after the adjuvant laser therapy than after conventional treatment.

#### 3. *Actinobacillus actinomycetem-comitans*:

Since A.a. was not able to be detected with the genomic DNA probes in any of the patients, the evaluations only refer to the bacterial count determined by the 16S-rRNA probes. (The manufacturer of the DNA probes was made aware of this phenomenon.)

A significant reduction in the bacterial count—compared to the initial stadium—is achieved both after conventional and the adjuvant laser treatment



over the entire period of observation. At first, the average bacterial count decreases strongly, especially after laser treatment, and then increases again from the third month onwards. The average bacterial counts with the laser method are always below the bacterial counts with the conventional method (see Diagram 1c). A statistically significant difference can be proved for measurements after one week to one month.

### Clinical Parameters:

Concerning the parameter pocket depth, no significant difference could be established in the direct comparison of both methods of treatment (cf. Diagram 2a). Whereas the examined periodontal pocket in the untreated quadrant showed unchanged haemorrhaging after probing over the entire study period, the total number of positive haemorrhage findings clearly decreased in the quadrant that had been treated with laser than in the one that had been treated conventionally. However, it increased again as from the third month after treatment (cf. Diagram 2b).

## Discussion

### Microbiological Parameters:

The qualitative and quantitative proof of the periodontopathogenic germs P.i., P.g. and A.a. should be used, if possible, to establish a diagnosis, make a ther-

**Diagram 2** Average measured depths of the periodontal pockets in mm (A) and number of such periodontal pockets examined here that haemorrhaged after probing (B) in the initial condition (0), i.e. 1, 3 and 6 months (1 M, 3 M, 6 M) after the particular therapy. Conventional curettage (■), additional Nd:YAG laser treatment (▲), no treatment (◆).



apeutic plan, control and fix the recall intervals. Numerous investigations have already shown that the detection of periodontopathogenic bacteria with DNA probes is superior to the other methods, like culture, antigen provocation tests or enzyme detection, regarding sensitivity and specificity.<sup>6,7,5</sup>

In this study, bacterial detection was performed with the help of both genomic DNA probes (DMDx/ PathoTek test) and 16S-rRNA probes.

Whilst both detection methods for P.i. and P.g. led to almost comparable results, A.a. was not able to be detected with genomic DNA probes in any treatment group, and was therefore determined with the 16S-rRNA probes only.

With all the three species that had been investigated here, the average values of the absolute bacterial count in the quadrants that had been treated with the adjuvant laser always lay below the corresponding values from the quadrants that had only been treated conventionally. There were statistically significant differences in favour of the laser method especially in the first measurements after treatment (after one week to one month).

The late recrudescence of the absolute bacterial counts through the recolonisation of the periodontal pockets was to be observed most clearly with A.a.—a pathogenic organism of which its refractory persistence against surgical and non-surgical attempts of elimination has been repeatedly reported about.<sup>8-11</sup> It proved to be a problematic germ in this study, too. According to this, a local elimination of A.a. only seems to persist over a period of about three months. After that, bacterial colonies are again formed, probably parting from other reservoirs in the oral cavity.<sup>12,13</sup> For this reason, one must try to make a systematic change in the recall system, meaning that the patients come in for a follow-up examination after every three months and, in the event of positive findings, receive laser treatment again on the affected periodontium.

Concerning the potent periodontopathogenic P.g., the reduction of the absolute bacterial count by a factor 30 compared with both methods can probably be explained by the fact that an effective reduction of the bacterial count already takes place in conventional treatment. The sensitive reaction of this germ to conventional methods of treating periodontitis gives rise to the supposition that it has its ecological recess mainly in the plaque and that a colonisation of the tissue does not occur. To grow, it requires obligative anaerobe conditions and prevailing conditions which could be created with the help of a preceding colonisation of the pocket with P.i. The aim of treatment of systematic periodontal therapy should be the reduction of the P.g. titre to below the level of detection.

#### Clinical Parameters:

The positive influence of a mechanical-instrumental periodontitis therapy on the clinical parameter probing depth has already been sufficiently investigated and is considered as generally recognised. The fear expressed in some studies that an additional laser treatment could lead to damage of the root cement and to periodontium—something that would appear first in an enlargement of the probing depth—has proved to be unfounded based on the results of this study. Supporting laser therapy has absolutely no negative influence on the probing depth; on the contrary, it led to a reduction of the probing depths.

A sulcus haemorrhage may not have a very high specificity as an inflammation criterion; however, owing to its high sensitivity, it does have sufficient meaningfulness as the earliest clinical sign for an inflammation, and is therefore essential for the early indication of a requirement for treatment. The reduction of a haemorrhagic tendency here with the help of an Nd:YAG laser confirms the effectiveness of this measure of treatment.

#### Conclusion

The results of this study prove that the application of the Nd:YAG laser in the treatment of periodontitis is—owing to its high bactericidal potency—a sensible measure that complements conventional therapy to reduce germs and to prevent a quick recolonisation of the affected periodontal pockets. The clinical findings, too, are influenced positively by the adjuvant application of laser.

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