Treatment of chronic periodontitis by laser and LED-PDT light

An in vivo study

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_Introduction

Periodontitis is a very common disease among adults, with approximately 80% having gingivitis and periodontitis, and 65% showing signs of periodontitis.¹ It is a major cause of tooth loss in adults and primarily considered an anaerobic bacterial infection, caused by the red complex species. Bacteria within a biofilm community, as well as enzymes, endotoxins, and other cytotoxic factors from these bacteria, lead to tissue destruction and the initiation of chronic inflammation.²

The conventional treatment entails the mechanical removal of calculus and the micro-organisms through scaling and root planning (SRP). Antimicrobial agents, used systemically or via local drug deliv-



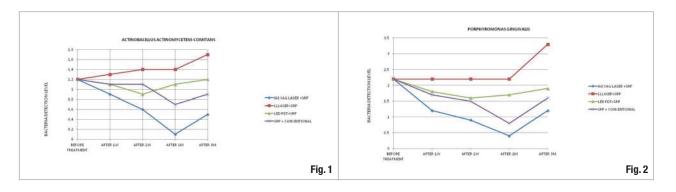
ery, further suppress the periodontal pathogens, thus increasing the benefits of conventional mechanical therapy. However, this conventional mechanical treatment of periodontitis is not always sufficient. Moreover, the emergence of resistant micro-organisms and a shift in the microflora after extended antimicrobial application limits their effect. Other approaches to the local delivery of antimicrobial agents have been investigated, including the use of high-energy pulsed lasers and photodynamic therapy (PDT).³

The Nd:YAG laser has been used in dentistry for nearly 20 years, primarily in minor surgery and endodontics. Nd:YAG laser energy is absorbed by tissues and it is this absorbance that allows surgical excision and coagulation of tissue.⁴ The 1,064 nm wavelength has a particular affinity towards melanin or other dark pigments. Therefore, darkly pigmented microbes are more sensitive to this laser and can be eliminated at very low power settings with no collateral damage to the adjacent tissue. Harris and Yessik⁵ developed a method for quantifying the efficacy of ablation of *Porphyromonas gingivalis in vitro*, using two different lasers.

Free-running pulsed Nd:YAG laser systems can generate high peak powers, at which the individual pulse power can reach several thousand watts. It allows this type of laser to deliver the required energy to the target tissue before the absorbed heat can dissipate from the treated area.

_Water-spray cooling

Although no comparative studies have been conducted on the need for water-spray cooling in both



diode and Nd:YAG laser procedures, water-spray can be said to be counter-productive from a biophysical point of view. Water is a good heat conductor and removes heat, whereas a thermal effect is desired. Cooling at the tissue surface is associated with the risk of deeper tissue necrosis. Therefore, water-spray should not be used in diode laser procedures.

For this reason, this study made use of an Nd:YAG laser without water and only with air spray.³

_LLLT lasers

The treatment of periodontitis with low-level laser therapy (LLLT) has not been common to date, and only a limited amount of literature exists. In a blind study by Qadri et al.,⁶ the clinical parameters, such as probing of the pocket depth, plaque index and gingival index, were more strongly reduced on the laser side than on the placebo side, with p < 0.01. The wavelengths used for LLLT have poor absorption in water, allowing for a penetration depth in soft and hard tissue ranging from 3 to 15 mm. As the energy penetrates tissues, it is scattered by both erythrocytes and micro-vessels. Because of this, both blood distribution and the network of micro-vessels in the tissue influence how the laser-light works, much like a bio-laser. LLLT lasers do not cut or ablate, but they use a photobiological process, which can have positive effects on periodontal healing and pain relief. LLLT is optimal for cells in a reduced oxygen environment.7

_Photodynamic therapy

PDT protocols use diode lasers or LED light with wavelengths that range from 635 to 690 nm, combined with a photosensitiser to eradicate sub-gingival microbes. PDT, which is also known as photo-radiation therapy, phototherapy or photochemotherapy, involves the use of a photoactive dye (photosensitiser), which is activated by exposure to light of a specific wavelength in the presence of oxygen. The transfer of energy from the activated photosensitiser to available oxygen results in the formation of toxic oxygen species, such as singlet oxygen and free radicals. The oral cavity is especially suitable for photodynamic antimicrobial chemotherapy (PACT) because it is relatively accessible for illumination.⁸

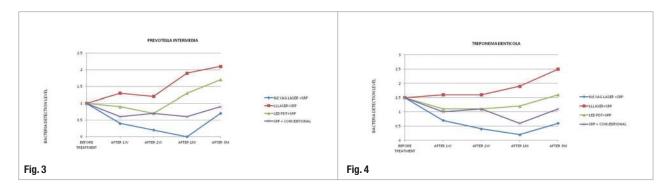
Dye concentration is a very important factor to be taken into consideration, since it results in a limited production of reactive oxygen (ROS) and requires a longer irradiation time. A high dye concentration works as an optical filter because of the resulting high absorption effect. The present study made use of a toluidine blue photosensitiser with a low concentration of 0.01 %, ensuring that soft-tissue irradiation and hard-tissue staining do not occur.

A semiconductor laser, such as the GaAlAs laser used in this study, has a coherence length of a few millimetres. This is very important, since the laser light produces constructive interference in tissue and consequent speckle formation. In contrast, LED light does not create speckles. These LED-light sources have a spectral width of 30 to 100 nm. LED reacts with cytochromes in the body. When cytochromes are activated, their energy levels increase, which stimulates tissue growth and regeneration.⁹

_Materials and methods

In January 2011, in Thessaloniki, Greece, 12 adults (seven women and five men), with an average age of 47 years (ranging from 29 to 68 years) were randomly assigned for treatment of the left or right side of the mouth. After the second week of the treatment, two patients had to leave the study because they had used antibiotics. The study was continued with ten patients, six women and four men. The patients were questioned about their systemic health status, their use of medication and smoking habits. In all of these cases, the inclusion criteria were periodontal pockets with probing depths deeper than 5 mm, with haemorrhagic findings and clinical signs of inflammation (swelling, secretion, etc.). Those individuals with a medical history of systemic disease requiring medication, those who had undergone antibiotic therapy within a three-month

Figs. 1 & 2_Change in detection levels of five bacteria from baseline (0) to one week and two weeks, to one month, and from one month to three months post-treatment.



Figs. 3–5_Change in detection levels of five bacteria from baseline (0) to one week and two weeks, to one month, and from one month to three months post-treatment. period preceding the study, and those with contagious diseases, as well as pregnant women and nursing mothers, were excluded.

Periodontal examination

At baseline, one trained examiner, who was masked to the test and control groups, measured the clinical periodontal parameters, including BOP (bleeding on probing) and PPD (probing pocket depth). These were measured with a graded periodontal probe (PerioWise, Premier Dental) at six sites. All teeth, except for the third molars, were registered.

Treatment protocols

The patients were randomly divided into four groups, which represented four different treatment modalities. At baseline (day 0), the patients assigned to group I received a one-hour session of full-mouth sub-gingival debridement, using a piezoceramic ultrasonic instrument (Piezon Master 400 with A+ PerioSlim tips, water coolant and power setting at 75%; EMS). The patients in group II received SRP with ultrasonic full-mouth debridement and then irradiation with a GaAlAs low-level laser at 830 nm (DIOBEAM, CMS Dental). The patients in group III received SRP with ultrasonic full-mouth debridement and then irradiation with a pulsed Nd:YAG laser at 1,064 nm (Genius). Finally, the patients in group IV received SRP with ultrasonic full-mouth debridement and then irradiation with the LED-PDT laser (FotoSan, CMS Dental).

Clinical parameter measurements

The PPD and BOP were measured for each group before the treatment, as well as one and three months post-treatment. Five clinical samples of gingival crevicular fluid were obtained from each patient and analysed with the DNA reverse-hybridisation laboratory process in the following order: (i) pretreatment; (ii) one week after the first laser or LEDlight session; (iii) one week after the second laser or LED-light session; (iv) one month post-treatment; and (v) three months post-treatment. A total of 200 microbiological samples were taken during the study period.

Laser parameters and lasers

The laser equipment used in this study was an Nd:YAG laser (1,064 nm), a GaAlAs low-level laser (830 nm) and a LED light (625–635 nm). The laser treatment was performed by inserting the optical fibre into the periodontal pocket, almost parallel to the tooth.

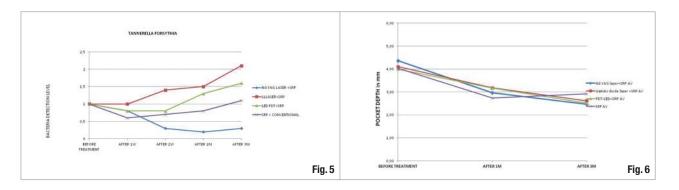
The Nd:YAG laser was used with the following settings: an average output of 4 W energy per pulse of 80 mJ; pulse width of 350 μ s and pulse repetition rate of 50 Hz; pulse peak power of 228 W; average power density at the fibre end of 1,415 W/cm²; and peak power density of 80,600 W/cm². Laser energy per treated tooth was 240 J. The fibre diameter was 600 μ s (0.002826 cm²). Only air cooling (+5 scale) was used during irradiation. The time spent on each tooth was 60 seconds.

The GaAlAs laser was used with the following power-intensity settings: the 8 J button was utilised and energy intensity was 63.5 J/cm². The buccal, palatal and lingual papillae were treated for 53.3 seconds per surface. The irradiated area was 0.25 cm².

The LED light used in this study had an output power of 2,000 mW/cm². The energy density (J/cm²) was not calculated because the energy was emitted not only from the tip, but also with considerable lateral emission. The sites were irradiated from both the buccal and lingual aspects.

Microbiological examination

Sterile paper points (ISO 40) were used for 30 seconds each to harvest sub-gingival plaque. The plaque was taken from the same site as the gingival crevicular fluid samples. After the paper point were collected from each quadrant, they were placed in sterile transport vials and sent to the laboratory for a thorough analysis. The sub-gingival microbiota were analysed using the checkerboard DNA-DNA hybridisation technique.³ Furthermore, the frequencies of positive sites and of sites with colony-forming units ≥103 were recorded. The following five micro-organisms were analysed: Aggregatibacter actinomycetemcomitans, P. gingivalis, Prevotella interme-



dia, Tannerella forsythensis and Treponema denticola ((AID Diagnostika GmbH, Periodontitis++).

Statistical methods

Commercially available software, such as Graph-Pad Prism 5 by GraphPad Software, was used for the statistical analysis. PPD and BOP were the primary clinical-outcome variables. Mean values and standard deviations (mean \pm SDs) for the clinical variables were calculated for each treatment, based on the subject as the statistical unit. The student's t-test was employed for continuous variables (clinical measurements), after the normality of the data distribution had been confirmed.

Similarly, the significance of the difference within each group, pre- and post-treatment, was evaluated with the paired-samples t-test. Differences were considered statistically significant when the p-value was less than 0.05.

The post-therapeutic data in each quadrant was checked against the initial data, using the paired t-test for statistically significant differences. Finally, the data of the quadrants additionally treated with laser was compared to the data of the conventionally treated quadrant as part of an unpaired t-test.

_Results

Microbiological results

All of the ten patients attended the baseline examination and the follow-up appointments. In the Nd:YAG laser- and SRP-treated group, it must be emphasised that *A. actinomycetemcomitans (A.a.)* levels were significantly reduced over the entire study period. It should also be noted that *A.a.* was reduced during the second week of treatment in the LED PDT- and SRP-treated group. Furthermore, the Gram-negative anaerobic bacteria were significantly reduced in the course of the study in the Nd:YAG laser group, in contrast with a lack of reduction for the other treatment groups. In the GaAlAs laser treated group, the microbiological samples showed an increase from moderate to higher detection levels. This result is in accordance with evidence in the literature that the low-level laser produces bacterial growth.¹⁰

Clinical results

All treatment groups showed significant decreases in PPD and BOP over the three-month post-treatment period. Baseline PPD was 4.37 (0.7) mm in the Nd:YAG quadrant, 4.10 (0.37) mm in the GaAlAs laser quadrant, 4.00 (0.4) mm in the LED-PDT quadrant and 4.03 (0.35) mm in the SRP quadrant. After three months of treatment, PPD was 2.47 (0.32) mm in the Nd:YAG quadrant, 2.62 (0.16) mm in the GaAlAs laser quadrant, 2.52 (0.29) mm in the LED-PDT quadrant and 2.92 (0.18) mm in the SRP quadrant. The reduction in PPD was significantly greater in the test group than the control group.

Baseline BOP for the Nd:YAG laser quadrant was 12.4. It decreased to five after one month and to zero after three months. For the GaAIAs laser quadrant, the baseline was 11.9, and BOP was 2.4 after one month and 0.5 after three months. In the LED-PDT quadrant, the baseline was 11.7, and BOP was 3.7 after one month and 0.8 after three months. For the SRP quadrant, the baseline was 12.3, and BOP was 4.9 after one month and 2.3 after three months.

For the PPD parameter, no significant difference could be established when comparing the four treatment methods used in this study directly. For the BOP index, the number of the haemorrhagic points clearly decreased in the quadrants treated with laser (Nd:YAG or GaAIAs laser) and with LED PDT, as compared with the one treated conventionally (Figs. 1–9).

_Discussion

The results in this study demonstrated that, when compared with the inflammation seen after nonsurgical treatment, additional treatment with the Nd:YAG laser, GaAIAs laser and LED-PDT light led to further reduction in gingival inflammation. Both the PPD and the number of BOP findings declined more **Fig. 6**_Change in PPD from baseline to one month and from one month to three months post-treatment in test and control teeth.

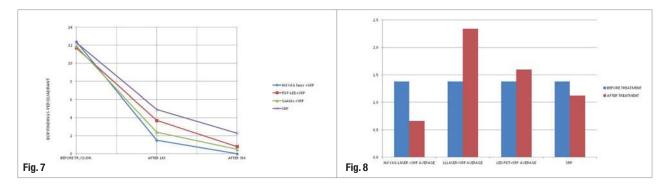


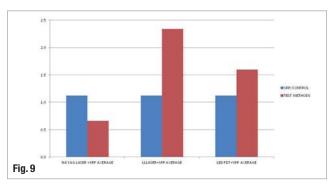
Fig. 7_Change in BOP findings per quadrant from baseline to one month and from one month to three months post-treatment in test and control teeth.

Fig. 8_The post-therapeutically established data in each treated quadrant was checked with the initial data for statistically significant differences with the help of a paired t-test. in the quadrant in which these additional treatments were performed.

The average values of the five microbial species that were investigated in this study were lower in the quadrants treated with Nd:YAG laser and SRP than in the quadrants that had been treated with GaAlAs laser and SRP, LED PDT and SRP, and conventional SRP. There were statistically significant differences in favour of the Nd:YAG laser method, especially in the first seven weeks. These findings confirm the findings from the study by Gutknecht et al.,¹¹ which demonstrated a reduction of specific micro-organisms in periodontal pockets when conventional treatment was supported by the use of an Nd:YAG laser.

Recurrence is reported in several studies and is attributed to cross-contamination from non-treated pockets and/or saliva.¹² In the present study, the Nd:YAG laser group was found to have a significant reduction in the levels of *A.a.*

The LED-PDT group also showed a reduction in *A.a.* levels, although this short-term decline was followed by a reappearance of the *A.a.* Both the GaAlAs laser and the LED-PDT groups, with toluidine blue as an adjunct to conventional SRP treatment, showed beneficial effects in clinical parameters like PPD and BOP, in comparison with the conventional SRP treatment.⁶ One explanation may be that laser irradiation reduces prostaglandin E2, or that it causes stimulation of cellular ATP. The GaAlAs laser also seems to have a stimulating effect on bacterial



growth, confirming similar findings in the scientific literature.¹³

However, certain pathogens, such as *A. actino-mycetemcomitans* and *P. gingivalis*, are particularly resistant to the effects of sub-gingival debridement. This has been linked to their ability to invade the pocket epithelium and underlying connective tissue.¹⁴

_Conclusion

The results of this study prove the positive role of coherent or non-coherent light irradiation as adjuncts to SRP in the non-surgical treatment of periodontitis. The ability of the Nd:YAG laser to reduce the levels of oral bacterial pathogens is much higher than that of LED PDT. Possibly one very important factor for the success of laser treatment is the regeneration of periodontal tissue, and it is best achieved by initially reducing the bacterial load.

LED PDT could be used as an alternative periodontal treatment after an initial treatment phase with the Nd:YAG laser. It should be limited to periods between treatment recalls. The short-term effect of LED PDT is sufficient to prevent a quick bacterial recolonisation of affected sites, especially in high-risk patients._

Editorial note: A complete list of references is available from the publisher.



Fig. 9_The data gathered in those quadrants that were additionally treated conventionally with the Nd:YAG laser, GaAIAs laser and LED PDT was compared with the data of the conventionally treated quadrant as a part of an unpaired t-test.

laser