J Clin Periodontol 2012; doi: 10.1111/j.1600-051X.2012.01925.x

Journal of Clinical Periodontology

Combined photoablative and photodynamic diode laser therapy as an adjunct to non-surgical periodontal treatment. A randomized split-mouth clinical trial

Marco Giannelli[1], Lucia Formigli[2], Luca Lorenzini[1] and Daniele Bani[2]

[1]Odontostomatologic Laser Therapy Center, Florence, Italy; [2]Department of Anatomy, Histology & Forensic Medicine, University of Florence, Florence, Italy

Giannelli M, Formigli L, Lorenzini L, Bani D. Combined photoablative and photodynamic diode laser therapy as an adjunct to non-surgical periodontal treatment. A randomized split-mouth clinical trial. J Clin Periodontol 2012; doi: 10.1111/j.1600-051X.2012.01925.x.

Abstract

Aim: Comparing the efficacy of photoablative and photodynamic diode laser in adjunct to scaling -root planing (SRP) and SRP alone for the treatment of chronic periodontitis.

Materials and Methods: Twenty-six patients were studied. Maxillary left or right quadrants were randomly assigned to sham-laser treatment + SRP or laser + SRP. This consisted of photoablative intra/extra-pocket de-epithelization with diode laser (λ = 810 nm), followed by single SRP and multiple photodynamic treatments (once weekly, 4–10 applications, mean ± SD: 3.7 ± 2.4) using diode laser (λ = 635 nm) and 0.3% methylene blue as photosensitizer. The patients were monitored at days 0 and 365 by clinical assessment (probing depth, PD; clinical attachment level, CAL; bleeding on probing, BOP) and at days 0, 15, 30, 45, 60, 75, 90, 365 by cytofluorescence analysis of gingival exfoliative samples taken in proximity of the teeth to be treated (polymorphonuclear leukocytes, PMN; red blood cells, RBC; damaged epithelial cells, DEC; bacteria).

Results: At day 365, compared with the control quadrants, the laser + SRP therapy yielded a significant ($p < 0.001$) reduction in PD (−1.9 mm), CAL (−1.7 mm) and BOP (−33.2% bleeding sites), as well as in bacterial contamination – especially spirochetes – and PMN and RBC shedding in the gingival samples ($p < 0.001$).

Conclusions: Diode laser treatment (photoablation followed by multiple photodynamic cycles) adjunctive to conventional SRP improves healing in chronic periodontitis patients.

Key words: cytofluorescence; diode laser; non-surgical/mechanical; periodontitis; photoablative; photodynamic; scaling and root planing; treatment/therapy

Accepted for publication 17 June 2012

Conflict of interest and source of funding

The authors declare that they have no conflict of interest.
The study was self-funded by the authors and/or their institution.

It is generally assumed that the success of chronic periodontitis treatment depends on abatement of periodontopathogenic microorganisms and/or their toxic by-products – such as lipopolysaccharide (LPS) – from the dental root surface and periodontal soft tissues, as well as neutralization of host pro-inflammatory cytokines. (Bascones et al. 2005, Tester et al. 2007, Giannobile 2008, Mombelli et al. 2011). It has been demonstrated that conventional scaling and root planing (SRP) do not completely remove periodontopathogens, especially in deep

periodontal pockets (Sbordone et al. 1990, Mombelli et al. 2000, Kawashima et al. 2007), and cannot prevent bacteria from spreading to periodontal soft tissues (Mombelli et al. 2011); SRP may even favour bacteraemic and endotoxaemic events (Forner et al. 2006, Lafaurie et al. 2007, Lee et al. 2008). The efficacy of the classical antiseptic/antibiotic approach (Goodson et al.1991, Renvert et al. 2006) is limited by the development of bacterial resistance (Ready et al. 2002, Ardila et al. 2010) which can account for unsatisfactory clinical outcomes (Bidault et al. 2007). Another crucial issue in the treatment of periodontitis consists in the fact that periodontopathogenic bacteria can penetrate into and persist in epithelial cells of the periodontal pockets and outer gingiva (Lamont & Yilmaz 2002, Tribble & Lamont 2010, Mishima & Sharma 2011), thus evading host immunity and conventional antimicrobial drugs. This can predispose to post-treatment re-colonization of periodontal tissues and hence, disease relapses and chronicization (Johnson et al. 2008, Ardila et al. 2010).

To overcome these issues, novel therapeutic approaches complementary to the classical strategies are required – one of which are medical lasers, which have aroused the interest of dental practitioners. The most commonly used devices in periodontics include semiconductor diode lasers, Nd:YAG laser (neodymium doped: yttrium, aluminium and garnet), Er:YAG laser (erbium doped:YAG) and carbon dioxide (CO_2) laser (Schwarz et al. 2008). Their wavelengths (λ) range from 630 to 10,600 nm. High-powered lasers (CO_2, Nd:YAG, diode λ = 810 –980) have been advocated for soft tissue surgery because they allow ablation/vaporization, haemostasis and sterilization (Moritz et al.1998, Miyazaki et al. 2003). Other authors have suggested Er:YAG lasers to remove plaque and calculus and to target bacteria in periodontal pockets (Schwarz et al. 2003a, b). Diode and Nd:YAG lasers have also been used for subgingival curettage and disinfection of periodontal pockets, although with uneven results (Cobb et al. 2010). In most cases, lasers have been used in photoablative (Pa) mode i.e. with high energy output. In recent years, photodynamic (Pd) therapy has taken hold among dental practitioners. This technique combines soft lasers at appropriate wavelength and photosensitizer substances, such as methylene blue, to produce singlet oxygen and free radicals with bactericidal properties (Braun et al. 2008, Lulic et al. 2009).

The current study was carried out to evaluate whether the combination of SRP and sequential Pa and Pd diode laser treatments benefited SRP alone in patients with chronic periodontitis. We used two diode lasers previously employed for periodontal therapy (Cobb et al. 2010), i.e. Gallium-Aluminium-Arsenide (GaAlAs) emitting at 810 nm wavelength and Indium Gallium-Aluminium-Phosphide (InGaAlP) emitting at 635 nm wavelength. The 810 nm diode laser was used in Pa mode for removal of the junctional, sulcular and outer gingival epithelium. We have demonstrated in a previous study that this device is superior to other dental lasers for such purposes (Giannelli et al. 2012). It also offers additional advantages, namely: (i) easy gingival reshaping; (ii) minimal pain at irradiation, reducing the need for local anaesthesia; (iii) excellent haemostasis, facilitating the subsequent SRP procedure. The 635 nm diode laser was used in Pd mode in combination with methylene blue with the purpose of attaining further microbial decontamination and chemical de-activation of harmful bacterial LPS (Kömerik et al. 2000, Giannelli et al. 2011). The null hypothesis to disprove was that no differences in the outcome variables, in terms of antimicrobial and anti-inflammatory effects and clinical periodontitis assessment parameters, exist between SRP alone and SRP + laser treatment after a 12-month follow-up.

Materials and Methods

Subjects

Twenty-eight patients, 16 men and 12 women, aged 25–65 (mean age 46.7), affected by chronic periodontitis were assessed for eligibility in a private periodontology clinic between January 2009 and April 2010. Twenty-six of these were consented to be included in the study (Fig. 1), which was performed in keeping with the consolidated standards of reporting trials (CONSORT) statement and complied with the guidelines of the Declaration of Helsinki, as amended in Edinburgh, 2008. This study was approved by the Ethical Committee of the Faculty of Medicine, University of Florence, Italy. Written informed consent for their enrolment in the study was given by all the subjects. Inclusion criteria were: (i) presence of at least two teeth with at least one site with pocket probing depth (PD) ranging between 4 and 10 mm in each upper maxillary quadrant with bleeding on probing (BOP), (ii) a minimum of five natural teeth in each studied quadrant. The exclusion criteria were: (i) history of systemic diseases (diabetes mellitus, cancer, HIV, metabolic and endocrine diseases), (ii) pregnancy or lactation, (iii) chronic high-dose steroid use, (iv) previous or current radiation or immunosuppressive therapies, (v) heavy smoking habit (>10 cigarettes/day), (vi) orthodontic treatment, (vii) extensive carious lesions, (viii) antibiotic medication during the 6 months preceding the study, (ix) class III tooth mobility, (x) heavy contamination by spirochetes and fungal pathogens on tongue and oral mucosa. The affected teeth of the lower mandibular quadrants were also treated, but not evaluated for this study.

Periodontal treatment randomization and allocation

In each patient, the upper quadrants were randomly allocated to one of the two regimens, e.g. laser + SRP or sham-laser treatment + SRP. Allocation concealment was performed by sequentially numbered opaque sealed envelopes (SNOSE). For each patient, the randomization envelope was opened immediately before the beginning of phase II treatment, as described below. Treatment assignment was registered by a non-clinical investigator (L.F.) and kept concealed from the clinical operator in charge of follow-up analyses until completion of this study.

Investigator features and blinding

A baseline periodontal clinical assessment was performed by an

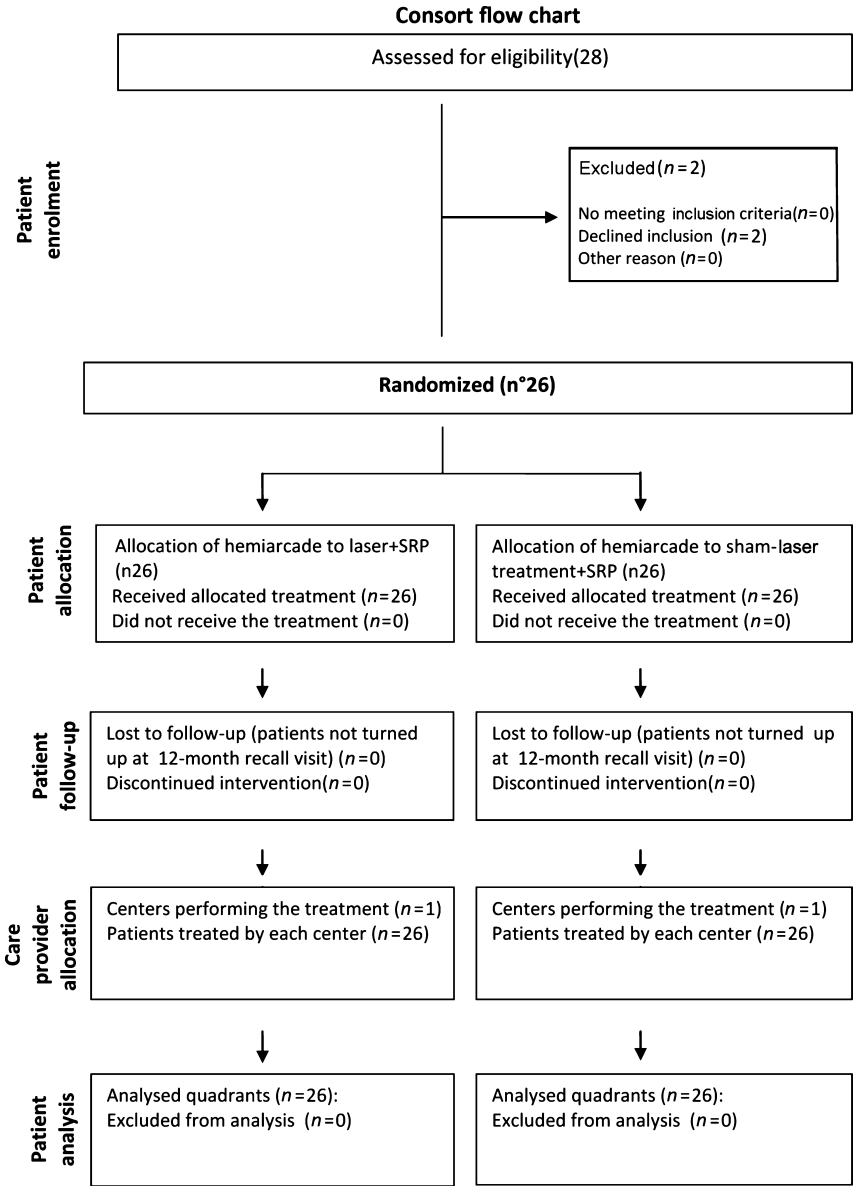

Fig. 1. Consolidated standards of reporting trials flow chart of the clinical study.

experienced dentist (L.L.), who was not involved in the patients' subsequent treatment, data collection and analysis. A total of eight periodontopathic subjects not included in the study were also recruited and used as standard reference by the examiner. The examiner recorded full-mouth PD and recessions at six sites per tooth (excluding the 3rd molars) in two different sessions on these subjects, using a conventional manual periodontal probe (PCP-12; Hu-Friedy, Chicago, IL, USA). Upon completion of all measurements, the intra-examiner repeatability for clinical attachment level (CAL) measurement was assessed. The examiner was deemed reliable when repeated measures of the same site were comprised in a range ±2 mm (Graziani et al. 2010). This investigator also collected follow-up data in a blinded fashion, being unaware of the treatments applied.

Operator features and blinding

The experienced operator who performed the treatments (M.G.) was not involved in any evaluation before or after his intervention. With the exception of the periodontal pocket chart, necessary to deliver the treatment, he was unaware of the previously recorded data.

Treatment phase I – Oral hygiene procedures

After admission to the study, the patients underwent:

- oral hygiene instructions and appropriate motivation; and
- full-mouth supragingival prophylaxis by ultrasound and/or hand instrumentation.

Periodontal clinical assessment

One week after professional oral hygiene, the patients were recalled to collect pre-study clinical data. This included evaluation of PD, CAL and BOP. CAL was calculated as PD plus recession (Rec, assumed as 0 whenever the cement-enamel junction was covered). Measurements were carried out at six sites per tooth and the values were averaged. BOP was assessed during PD assay by evaluating the presence or absence of bleeding, for more than 30 s, after challenging the pocket with the periodontal probe. The test and control areas comprised teeth 11–16 and 21–26. The test time points were day 0 (before therapy) and day 365 (after therapy).

Periodontal cytodiagnostic assessment

Cytological assay has become a common technique for the staging of chronic periodontitis (Filoche et al. 2007). In the patients under study, exfoliative samples were taken with a sterile microcurette (Rudney et al. 2001) at the free gingival margin of the teeth to be treated at day 0 (before therapy) and at days 15, 30, 45, 60, 75, 90 and 365 after the therapy. Samples, containing both cells from gingival epithelium and blood cells and bacteria contained in crevicular fluid squeezed out from the pocket during the manoeuvre, were processed for cytofluorescent staining using the LIVE/DEAD BacLight^{TM} bacterial viability kit (Invitrogen Molecular Probes, Milan, Italy), as described (Giannelli et al. 2010). This method, originally developed for microbiological purposes to monitor the viability of bacteria by assessing plasma membrane integrity (Berney et al. 2007), has been applied to cytodiagnostic purposes in dental patients (van der Mei et al. 2006, Filoche et al. 2007, Tomás

et al. 2009). It yields a broad range of diagnostic information, such as the amount and viability of contaminating bacteria, including cocci, bacilli and spirochetes, as well as of inflammatory polymorphonuclear leukocytes (PMN), erythrocytes (RBC) and damaged epithelial cells (DEC) (Giannelli et al. 2010, 2012). Briefly, the samples were smeared on a histological slide, fixed in 90% ethanol, air-dried and stained with 1 ml of the fluorescent LIVE/DEAD BacLight™ solution for 2 min at 37°C. After thorough rinsing in distilled water, samples were mounted in oil and immediately observed under a Leica 4000 B fluorescent microscope (Leica Microsystems, Milan, Italy). Viability is differentiated based on membrane integrity. Bacteria with damaged membranes are deemed as non-viable and stain red with the propidium iodide component. Bacteria with intact membranes are deemed viable and stain green with the SYTO9 component, resulting in differential staining. The parameters were semi-quantitatively scored according to Giannelli et al. (2010), as reported in Table 1.

Treatment phase II – Photoablative laser therapy and SRP

After data collection, patients were recalled to undergo phase II treatment. Each patient underwent two parallel treatments: the teeth on the test maxillary quadrant were given laser treatment + SRP, whereas those of the contra-lateral control quadrant were given sham-laser treatment + SRP. The gingival mucosa was subjected to Pa treatment with a diode laser operating at 810 nm wavelength (1 W output power, continuous wave, 66.7 J/cm^2), equipped with a 0.6 mm optical fibre (4 × 4 Dental Laser™; General Project, Montespertoli, Italy). Irradiation was performed in contact mode, the fibre tip touching the gingiva, to remove the junctional, sulcular and outer gingival epithelium (approx. 5 mm from the gingival margin) all around the teeth (Fig. 2A). To minimize gingival damage, the tip was moved at a constant speed of 2.5 mm/s. The fibre end was controlled at every irradiation to check for a carbonized tip (hot tip), required to generate enough thermal energy to cause tissue vaporization at the incision line. When the fibre comes in contact with tissue and blood, the debris which immediately accumulate on its tip absorb the intense infrared laser energy, thus heating the tip and carbonizing the debris and the optic fibre end. As laser energy continues to be absorbed by carbon deposits, the tip reaches a red hot temperature (~760°C), causing tissue vaporization (Bornstein 2004). Excess carbonized debris was removed with wet gauze. The $\lambda = 810$ nm diode laser, used with the hot tip technique, was chosen because of its low tissue penetration, which permitted complete removal of the gingival epithelium contaminated by intra-cellular periodontopathogens with minimal injury to the underlying lamina propria (Giannelli et al. 2012). Pa treatment was performed under thermographic monitoring, with threshold set at 80°C on the target (Bornstein 2004), to avoid undesired heat-induced tissue damage. The Pa diode laser treatment was not extended to the dental root tissues because it has been found ineffective in removing mineralized deposit from the root surface (Schwartz et al. 2003b). Eye protection of the operator, assistant and patients was assured by wearing safety glasses. The complete irradiation parameters are reported in Table 2. Local anaesthesia (Articain HCl; Ultracain, Frankfurt, Germany) was given on demand to a single patient on the laser-treated quadrant, and to 15 patients on the control quadrants before SRP.

Sham-laser treatment consisted of the same manual operations performed with the laser switched off. At the end of the Pa or sham treatments, conventional SRP was performed using Gracey curettes (Hu-Friedy).

The operator (M.G.) was not involved in the analysis of follow-up data.

Table 1. Severity scoring criteria*

SCORE	0	1	2	3
PMN, RBC	Absent	<5	5–10	>10
DEC	Normal	Aberrant shape	Plasma membrane rupture, vacuolation	Conglutination, vacuolation cell debris
Cocci, bacilli, spirochetes	Absent	< 10	10–30	> 30

*Modified from Giannelli et al. (2010).
Numbers are intended per microscopical field.
DEC, damaged epithelial cells; PMN, polymorphonuclear leukocytes; RBC, erythrocytes.

Treatment phase III – Photodynamic mode

At the next clinical session (approximately once weekly), the laser-irradiated mucosa was subjected to Pd treatment with a diode laser operating at 635 nm wavelength (100 mW output power, continuous wave), equipped with a 0.6 mm optic fibre (4 × 4 Dental Laser; General Project, Montespertoli, Italy). In this instrument, the Pd laser source was assembled with the Pa one in the same console. The periodontal tissue, including the pocket and surrounding mucosa, and the dental root were rinsed with the photosensitizer agent methylene blue (0.3% w/v in water). After 5 min., the inner and outer pocket mucosa around each tooth and the dental root were laser-irradiated. The optical fibre was gently introduced into the pocket and moved circularly in deep-to-cervical direction, or moved smoothly over the outer gingiva with a 2.5 mm/s speed (average time per tooth: 1 min. inside and 1 min. outside the pocket). This procedure did not require any local anaesthesia. Pd treatment was continued until normalization of the cytodiagnostic parameters, especially PMN (range: 4–10 applications). Additional data are reported in Table 2. Eye protection of the operator, assistant and patients was assured by wearing safety glasses. Sham-laser treatment consisted of methylene blue rinsing and identical manual operations performed with the laser switched off.

Post-treatment instructions

Patients were instructed to discontinue toothbrushing on the day of Pa therapy to prevent mechanical

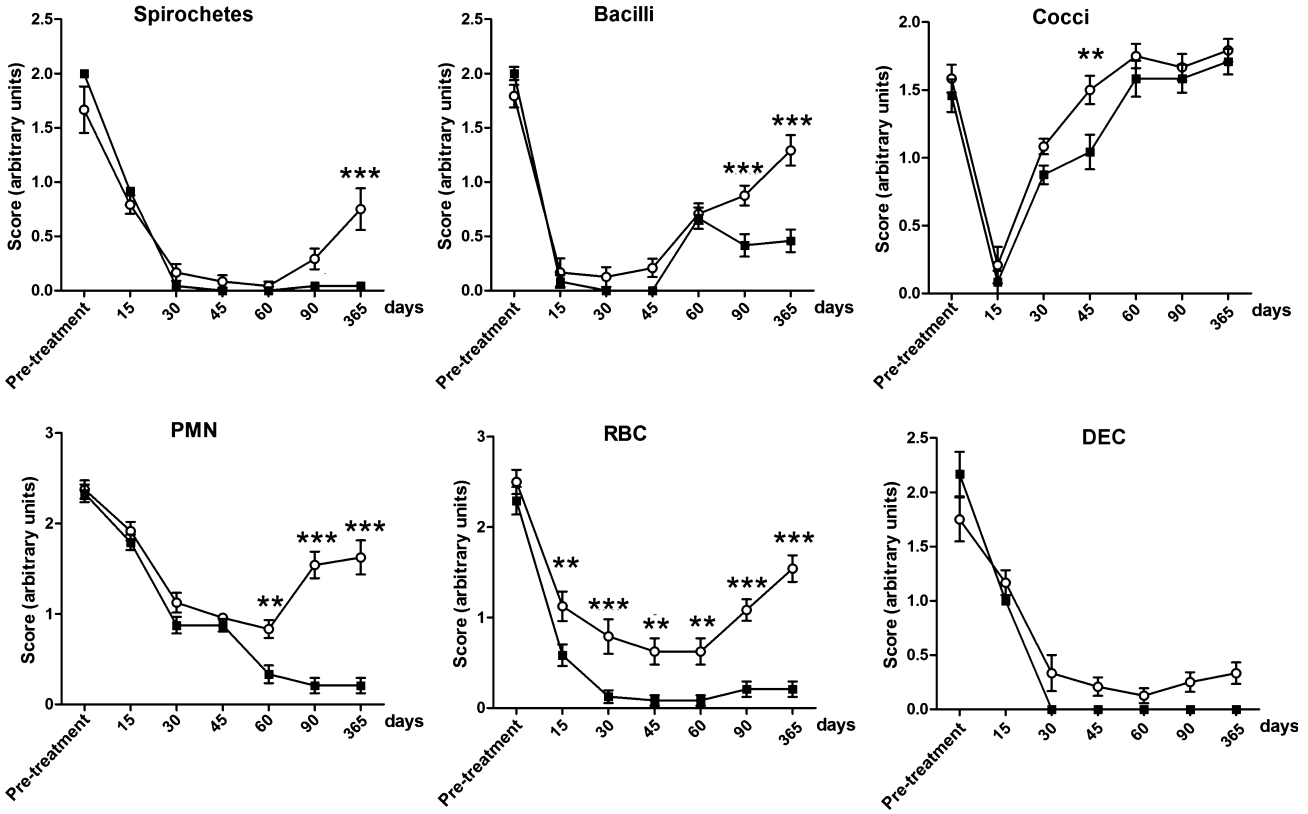

Fig. 2. Time course of cytodiagnostic parameters evaluating microbial contamination (upper panels), polymorphonuclear leukocytes (PMN), red blood cells (RBC) and damaged epithelial cells (DEC) in the patients receiving sham-laser treatment + SRP (○) or laser + SRP (■) during 1-year follow-up. Statistically, the interaction between time points and treatments was significant for all the parameters (two-way repeated measures ANOVA: $p < 0.001$). Differences at individual time points (paired *t*-test and Bonferroni's multiple comparison test): $^{**}p < 0.01$, $^{***}p < 0.001$.

trauma at the treated sites and facilitate re-epithelization. From day 2, normal personal tooth hygiene with a toothbrush and inter-proximal instruments was encouraged. Local use of chlorhexidine digluconate was not prescribed.

Statistical analysis

The subject's quadrant was assumed as a test unit for statistical comparison. For the clinical parameters, each test unit resulted from the average of six measurements per tooth and a minimum of five teeth per quadrant. For the cytodiagnostic parameters, each test unit was the average of five sampling sites per quadrant. Values were expressed as means ± SEM. The clinical parameters were compared by Student's *t*-test for paired values. The cytodiagnostic parameters, which varied depending on treatment and time, were first analysed by two-way repeated measures ANOVA to assess whether the interaction between the two variables was significant. If so, differences between each time point were assessed by paired *t*-test followed by Bonferroni multiple comparison test (Lesaffre et al. 2007). A *p*-value ≤ 0.05 was considered significant.

Results

All the enrolled patients successfully completed the study. A total of 150 teeth per experimental group was evaluated and compared. The values of the clinical parameters (PD, CAL, BOP) assayed before the treatments (day 0) and after a 365-day follow-up (corresponding to 900 measurements per each parameter) are reported in Table 3. We found that both sham-laser treatment + SRP and laser + SRP caused a significant improvement of these parameters compared with the pre-treatment baseline values. In comparison with the teeth of the control quadrant, laser + SRP therapy significantly reduced ($p < 0.001$) PD (−1.9 mm with respect to 4.0 ± 0.1 mm in the controls), CAL (−1.7 mm with respect to 4.8 ± 0.2 mm in the controls) and BOP (−33.2% with respect to 37.0 ± 0.9% in the controls).

The cytodiagnostic assay used to monitor the severity and progression of periodontal disease, was performed before the therapy (day 0) and at seven time points (15, 30, 45, 60, 47, 90, 365 days) during follow-up. The results obtained are shown in Fig. 2. This assay revealed a strong, significant reduction ($p < 0.001$) in PMN shedding, an index of periodontal inflammation, in both the treatment groups at a 30-day follow-up. In the longer follow-up, the inflammatory infiltrate tended to rise progressively in the sham-laser treatment + SRP quadrants, whereas it decreased further in the laser + SRP-treated ones. Stable reduction (PMN < 5) or even

Table 2. Laser irradiation parameters

Laser beam characteristics		
	Diode 810 photoablative mode	Diode 635 photodynamic mode
Wavelength	810 nm	635 nm
Irradiation mode	Continuous wave (CW)	Continuous wave (CW)
Power	1 W	100 mW
Fibre diameter	0.6 mm	0.6 mm

Surface treatment data			
Treatment mode *	Contact (gingival pocket internal + external)	Contact (gingival pocket internal)	Non-contact: 1 mm (gingival pocket external)
Laser spot at target diameter/area	0.6 mm/0.3 mm^2	0.6 mm/0.3 mm^2	1.1 mm/0.9 mm^2
Fibre movement speed	2.5 mm/s	2.5 mm/s	2.5 mm/s
Power density	353.4 W/cm^2	35.3 W/cm^2	11.6 W/cm^2
Total energy density (fluence)	66.7 J/cm^2	6.7 J/cm^2 each passage	3.8 J/cm^2 each passage

*Laser treatments were performed as follows:
For photoablative treatment, contiguous tissue strips were removed moving the fibre tip for 2 s on the gingival surface, thus progressively ablating the junctional, sulcular and outer pocket epithelium.
For photodynamic treatment, the inner pocket was irradiated by repeated passages of the fibre tip in contact mode, 2 s each, whereas the outer pocket was irradiated by repeated passages of the fibre tip in non-contact mode, 2 s each.

Table 3. Clinical parameters in the different groups

Index/treatment	(±SEM)	365-day follow-up (±SEM)	*p*-value
PD (mm)			
Sham + SRP	4.9 ± 0.1	4.0 ± 0.1	<0.001
Laser + SRP	5.1 ± 0.1	2.1 ± 0.1	<0.001
p-value	n.s.	<0.001	
CAL (mm)			
Sham + SRP	5.6 ± 0.2	4.8 ± 0.2	<0.001
Laser + SRP	5.6 ± 0.2	3.1 ± 0.2	<0.001
p-value	n.s.	<0.001	
BOP (%)			
Sham + SRP	68.9 ± 2.9	37.0 ± 0.9	<0.001
Laser + SRP	69.4 ± 3.2	3.8 ± 1.1	<0.001
p-value	n.s.	<0.001	

Significance of differences within and between the groups at different time points by Student's *t*-test for paired values.
BOP, bleeding on probing; CAL, clinical attachment level; PD, pocket probing depth.

normalization (PMN = 0) of the PMN parameter with laser + SRP treatment was achieved upon repeated Pd applications, ranging from 4 (six patients) to 10 (one patient) (mean ± SD: 3.7 ± 2.4).

Measurement of RBC, a bleeding index, indicated that laser + SRP induced a marked improvement of this parameter from 30 days onwards, which was significantly higher than that obtained with sham-laser treatment + SRP ($p < 0.001$). A similar trend was shown by the measurement of DEC, an index of gingival epithelial injury, but the differences did not reach statistical significance.

Semi-quantitative assessment of bacteria in the cytodiagnostic samples showed that both treatments caused a rapid abatement of microbial contamination, reaching a minimum at 15–30 days. Spirochetes, a parameter of periodontal disease severity, were similarly reduced by both treatments at a 60-day follow-up. In the longer follow-up, spirochetes tended to regrow in the sham-laser treatment + SRP group, whereas they remained undetectable in the laser + SRP group ($p < 0.001$). Bacilli showed a similar trend as spirochetes, except for a progressive regrowth from day 60 onwards observed in both the test and control quadrants. Notably, this phenomenon was less prominent in the laser + SRP quadrants than in the sham-laser treatment + SRP ones ($p < 0.001$). Cocci were substantially abated by both treatments at day 15 and their number increased again thereafter. No major differences were noted between the laser + SRP group and the sham-laser treatment + SRP group.

Finally, it should be mentioned that the post-treatment course was uneventful in all patients and that no complications, such as abscesses or infections, were observed throughout the follow-up. Moreover, all patients but one perceived little or no discomfort during Pa treatment, allowing the operator to not administer local anaesthesia.

Discussion

This study demonstrates the clinical efficacy of a comprehensive periodontal treatment protocol for chronic periodontitis, based on diode lasers used sequentially in Pa and Pd modes in combination with conventional SRP. In comparison with SRP alone by means of hand instruments, the laser + SRP treatment led to a significant improvement of all the clinical parameters assayed after a 12-month follow-up. Moreover, in the laser + SRP-treated quadrants, the patients consistently showed a rapid and persistent abatement of contamination by the most aggressive periodontopathogens, such as spirochetes (Colombo et al. 2007, Visser & Ellen 2011), as well as a remarkable reduction in the amount of RBC and PMN, whose presence in periodontal tissues is related to the severity of the local inflammatory process (Giannelli et al. 2010, Bhadbhade et al. 2012). Indeed, successful elimination of infiltrating leukocytes, which represent a source of harmful products, such as reactive oxygen species, inflammatory mediators and matrix-degrading enzymes, from diseased periodontal tissues has been shown to be a critical step for tissue healing and repair (Nussbaum & Shapira 2011). In this

context, the marked reduction in PMNs observed in the laser + SRP-treated quadrants may depend on the ability of the diode laser, applied sequentially in Pa and Pd modes, to modulate the tissue levels of molecules involved in tissue inflammation and remodelling, such as IL-1β, cyclooxygenase-2, matrix metalloprotease-8, PDGF and TGF-β (Nomura et al. 2001, de Paula Eduardo et al. 2010) and to blunt the expression of endothelial leucocyte adhesion molecule ICAM-1 (Giannelli et al. 2012). Moreover, based on previous observations that LPS released by periodontopathogenic Gram-negative bacteria can adhere to the root surface, persist in periodontal soft tissues after SRP and contribute to chronic inflammation (Nair et al. 1996), we suggest that the strong reduction in periodontal PMNs could also be due to laser-mediated de-activation of LPS, as we have previously shown (Giannelli et al. 2011). Indeed, the Pd approach takes advantage of the photochemical properties of methylene blue, namely low molecular weight, positive charge and hydrophilia, which make it able to interact with negatively charged LPS and deactivate it. Moreover, the same characteristics allow methylene blue to easily enter Gram-negative bacteria through the porin-protein channels of the outer membrane (Fontana et al. 2009); upon photo-activation with the 635 nm wavelength diode laser, methylene blue releases oxidizing metabolites which exert potent LPS-deactivating and antiseptic effects (Fontana et al. 2009).

We have also shown that periodontal cocci and bacilli tend to progressively regrow during the follow-up period in both the laser + SRP and sham-laser treatment + SRP-treated quadrants. As these morphologically identified classes of microorganisms have been shown to include both active and quiescent periodontopathogens, such as *Porphyromonas gingivalis* and *Aggregatibacter actinomycetemcomitans*, as well as normal components of the buccal microenvironment, such as Gram-positive bacteria of the genera *Streptococcus* and *Actinomyces* (Giannelli et al. 2010), their re-growth after the periodontal treatments is probably not relevant to the therapy outcome, because it may merely indicate replacement of periodontopathogens by normal oral microbial flora, as also reported in previous studies (Lavanchy et al. 1987, Sbordone et al. 1990).

Cytodiagnosis may play a key role in the assessment of periodontal disease: through detection and quantification of blood cells, epithelial cells and bacteria, it can help evaluating the stage and nature of adaptative reactions of the periodontium in response to pathogenic microbial flora (Filoche et al. 2007). The fluorescent viability assay used in this study has been applied to microorganisms from different ecosystems, including oral bacteria (van der Mei et al. 2006, Filoche et al. 2007, Tomás et al. 2009). Albeit not being a standard method in periodontology, this assay allowed us to perform an objective assessment of the patients under study, as previously reported (Giannelli et al. 2010, 2012).

From a practical viewpoint, during Pa treatment it is important to carefully discriminate between laser-ablated and untreated tissue to avoid repeated irradiation of the gingival mucosa. Correct Pa treatment can prevent thermal damage of deep tissues while ensuring complete removal of the surface epithelium (Giannelli et al. 2012) purportedly contaminated by intra-cellular periodontopathogens (Rudney et al. 2001, Johnson et al. 2008). This may reduce the risk for bacterial re-growth (Mombelli et al. 2000).

Despite these findings and the other encouraging reports from in vitro experiments, animal studies and randomized clinical trials (Moritz et al.1998, Yilmaz et al. 2002, Braun et al. 2008, Braham et al. 2009, de Paula Eduardo et al. 2010), there is still controversy regarding the actual clinical efficacy of laser treatments compared with conventional SRP for the therapy of chronic periodontitis (Christodoulides et al. 2008, Polansky et al.2009). Much debate stems from comparing non-homogeneous categories, in terms of different laser types and wavelengths, wide variations in laser parameters and differences in the therapeutic design and indications (Aoki et al. 2004, Schwarz et al. 2008, Cobb et al. 2010). Another issue of controversy is related to the fact that most of the previous studies limit the laser treatment to periodontal pocket curettage (Lin et al. 2011), which has been recently stated to be substantially ineffective in a consensus report of the AAP Board of Trustees (2011). As a matter of fact, no standardized protocols for laser therapy in periodontology have been established yet. Thus, the results of the different studies are hardly comparable because they are largely dependent on individual skills and background photobiological knowledge of the different investigators. Our findings cannot be directly compared with those of previous studies because – to the best of our knowledge – this is the first study to combine Pa and Pd laser treatment as an adjunct to SRP. Notwithstanding this, our findings support previous reports that treatment with Pa (Moritz et al. 1998, Andreana 2005, Lin et al. 2011) or Pd (Polansky et al. 2009, Cappuyns et al. 2011, Ge et al. 2011) diode lasers can afford significant advantages over SRP alone in patients with chronic periodontitis. However, our study does not allow us to discriminate which diode laser treatment, Pa or Pd, contributes more to improve the clinical outcome. Conceivably, gingival epithelial removal by Pa irradiation and further periodontal decontamination by repeated Pd applications could be considered as synergistic treatments and may both be required for optimum clinical results.

The use of Pa laser can raise safety concerns, because its effects on gingival tissues are difficult to fine-tune. In this view, accurate setting of the irradiation parameters is crucial to reduce the possibility of iatrogenic gingival damage, e.g. due to overheating. Another key issue to be considered is that the "hot tip" phenomenon reduces tissue penetration of the laser beam and accounts for a different laser-tissue interaction than that occurring when irradiation is performed with a non-carbonized tip. This notion should be considered by dental operators to achieve safe and predictable Pa treatment with diode lasers.

As a concluding remark, the favourable characteristics of diode lasers used sequentially in Pa and Pd modes as adjuncts to conventional SRP may be considered valuable

tools for the treatment of chronic periodontitis. The post-treatment course was uneventful in all our cases and no complications arose throughout the follow-up. Most of our patients perceived very little discomfort during treatment, even in the absence of local anaesthesia, and had an overall preference for the laser modality, as also recently reported elsewhere (Lin et al. 2011). This indicates that the laser + SRP protocol is safe and well tolerated.

Acknowledgements

The authors gratefully acknowledge Mrs. Tiziana Fossi for skilful help in clinical data collection and patient monitoring, Dr. Eng. Massimo Lasagni for technical support, General Project, (Montespertoli, Italy) for kindly providing the 4 × 4 Dental Laser™ instrument, Dr. Mary Forrest for careful English language revision.

References

AAP Board of Trustees (2011) American Academy of Periodontology statement on the efficacy of lasers in the non-surgical treatment of inflammatory periodontal disease. *Journal of Periodontology* **82**, 513–514.

Andreana, S. (2005) The use of diode lasers in periodontal therapy: literature review and suggested technique. *Dentistry Today* **24**, 132–135.

Aoki, A., Sasaki, K. M., Watanabe, H. & Ishikawa, I. (2004) Lasers in nonsurgical periodontal therapy. *Periodontology* **2000**, 59–97.

Ardila, C. M., Granada, M. I. & Guzmán, I. C. (2010) Antibiotic resistance of subgingival species in chronic periodontitis patients. *Journal of Periodontal Research* **45**, 557–563.

Bascones, A., Noronha, S., Gómez, M., Mota, P., Gónzalez Moles, M. A. & Villarroel Dorrego, M. (2005) Tissue destruction in periodontitis: bacteria or cytokines fault? *Quintessence International* **36**, 299–306.

Berney, M., Hammes, F., Bosshard, F., Weilenmann, H. U. & Egli, T. (2007) Assessment and interpretation of bacterial viability by using the LIVE/DEAD Baclight Kit in combination with flow cytometry. *Applied Environmental Microbiology* **73**, 3283–3290.

Bhadbhade, S. J., Acharya, A. B. & Thakur, S. (2012) Correlation between probing pocket depth and neutrophil counts in dental plaque, saliva, and gingival crevicular fluid. *Quintessence International* **43**, 111–117.

Bidault, P., Chandad, F. & Grenier, D. (2007) Risk of bacterial resistance associated with systemic antibiotic therapy in periodontology. *Journal of Cananadian Dental Association* **73**, 721–725.

Bornstein, E. (2004) Near-infrared dental diode lasers. Scientific and photobiologic principles and applications. *Dentistry Today* **23**, 102–108.

Braham, P., Herron, C., Street, C. & Darveau, R. (2009) Antimicrobial photodynamic therapy may promote periodontal healing through multiple mechanisms. *Journal of Periodontology* **80**, 1790–1798.

Braun, A., Dehn, C., Krause, F. & Jepsen, S. (2008) Short-term clinical effects of adjunctive antimicrobial photodynamic therapy in periodontal treatment: a randomized clinical trial. *Journal of Clinical Periodontology* **35**, 877–884.

Cappuyns, I., Cionca, N., Wick, P., Giannopoulou, C. & Mombelli, A. (2011) Treatment of residual pockets with photodynamic therapy, diode laser, or deep scaling. A randomized, split-mouth controlled clinical trial. *Lasers in Medical Science*. Nov 22 [Epub ahead of print].

Christodoulides, N., Nikolidakis, D., Chondros, P., Becker, J., Schwarz, F., Rössler, R. & Sculean, A. (2008) Photodynamic therapy as an adjunct to non-surgical periodontal treatment: a randomized, controlled clinical trial. *Journal of Periodontology* **79**, 1638–1644.

Cobb, C. M., Low, S. B. & Coluzzi, D. J. (2010) Lasers and the treatment of chronic periodontitis. *Dental Clinics of North America* **54**, 35–53.

Colombo, A. V., da Silva, C. M., Haffajee, A. & Colombo, A. P. (2007) Identification of intracellular oral species within human crevicular epithelial cells from subjects with chronic periodontitis by fluorescence *in situ* hybridization. *Journal of Periodontal Research* **42**, 236–243.

Filoche, S. K., Coleman, M. J., Angker, L. & Sissons, C. H. (2007) A fluorescence assay to determine the viable biomass of microcosm dental plaque biofilms. *Journal of Microbiological Methods* **69**, 489–496.

Fontana, C. R., Abernethy, A. D., Som, S., Ruggiero, K., Doucette, S., Marcantonio, R. C., Boussios, C. I., Kent, R., Goodson, J. M., Tanner, A. C. & Soukos, N. S. (2009) The antibacterial effect of photodynamic therapy in dental plaque-derived biofilms. *Journal of Periodontal Research* **44**, 751–759.

Forner, L., Neilson, C. H., Bendtzen, K., Larson, T. & Holmstrup, P. (2006) Increased plasma levels of IL-6 in bacteremic periodontitis patients after scaling. *Journal of Clinical Periodontology* **33**, 724–729.

Ge, L., Shu, R., Li, Y., Li, C., Luo, L., Song, Z., Xie, Y. & Liu, D. (2011) Adjunctive effect of photodynamic therapy to scaling and root planing in the treatment of chronic periodontitis. *Photomedicine and Laser Surgery* **29**, 33–37.

Giannelli, M., Bani, D., Viti, C., Tani, A., Lorenzini, L., Zecchi-Orlandini, S. & Formigli, L. (2012) Comparative evaluation of the effects of different photoablative laser irradiation protocols on the gingiva of periodontopathic patients. *Photomedicine and Laser Surgery* **30**, 222–230.

Giannelli, M., Formigli, L. & Bani, D.. (2010). A novel cytodiagnostic fluorescence assay for the diagnosis of periodontitis. In: Yamamoto, S. L. (ed.). *Periodontal Disease: Symptoms, Treatment and Prevention*, pp. 137–151. Nova Publishers: Hauppage, NY.

Giannelli, M., Pini, A., Formigli, L. & Bani, D. (2011) Comparative *in vitro* study among the effects of different laser and LED irradiation protocols and conventional chlorhexidine treatment for deactivation of bacterial lipopolysaccharide adherent to titanium surface. *Photomedicine and Laser Surgery* **29**, 573–580.

Giannobile, W. V. (2008) Host-response therapeutics for periodontal diseases. *Journal of Periodontology* **79**(Suppl. 8), 1592–1600.

Goodson, J. M., Tanner, A., McArdle, S., Dix, K. & Watanabe, S. M. (1991) Multicenter evaluation of tetracycline fiber therapy. III. Microbiological response. *Journal of Periodontal Research* **26**, 440–451.

Graziani, F., Cei, S., Tonetti, M., Paolantonio, M., Serio, R., Sammartino, G., Gabriele, M. & D'Aiuto, F. (2010) Systemic inflammation following non-surgical and surgical periodontal therapy. *Journal of Clinical Periodontology* **37**, 848–854.

Johnson, J. D., Chen, R., Lenton, P. A., Zhang, G., Hinrichs, J. E. & Rudney, J. D. (2008) Persistence of extracrevicular bacterial reservoirs after treatment of aggressive periodontitis. *Journal of Periodontology* **79**, 2305–2312.

Kawashima, H., Sato, S., Kishida, M. & Ito, K. (2007) A comparison of root surface instrumentation using two piezoelectric ultrasonic scalers and a hand scaler *in vivo*. *Journal of Periodontal Research* **42**, 90–95.

Kömerik, N., Wilson, M. & Poole, S. (2000) The effect of photodynamic action on two virulence factors of gram-negative bacteria. *Photochemistry and Photobiology* **72**, 676–680.

Lafaurie, G. I., Mayorga-Fayad, I., Torres, M. F., Castillo, D. M., Aya, M. R., Barón, A. & Hurtado, P. A. (2007) Periodontopathic microorganisms in peripheric blood after scaling and root planing. *Journal of Clinical Periodontology* **34**, 873–879.

Lamont, R. J. & Yilmaz, O. (2002) In or out: the invasiveness of oral bacteria. *Periodontology* **2000**, 61–69.

Lavanchy, D., Bickel, M. & Baehni, P. (1987) The effect of plaque control after scaling and root planing on the subgingival microflora in human periodontitis. *Journal of Clinical Periodontology* **14**, 295–299.

Lee, M. K., Ide, M., Coward, P. Y. & Wilson, R. F. (2008) Effect of ultrasonic debridement using a chlorhexidine irrigant on circulating levels of lipopolysaccharides and interleukin-6. *Journal of Clinical Periodontology* **35**, 415–419.

Lesaffre, E., Garcia Zattera, M. J., Redmond, C., Huber, H. & Needleman, I. (2007) Reported methodological quality of split-mouth studies. *Journal of Clinical Periodontology* **34**, 756–761.

Lin, J., Bi, L., Wang, L., Song, Y., Ma, W., Jensen, S. & Cao, D. (2011) Gingival curettage study comparing a laser treatment to hand instruments. *Lasers in Medical Science* **26**, 7–11.

Lulic, M., Leiggener Görög, I., Salvi, G. E., Ramseier, C. A., Mattheos, N. & Lang, N. P. (2009) One-year outcomes of repeated adjunctive photodynamic therapy during periodontal maintenance: a proof-of-principle randomized-controlled clinical trial. *Journal of Clinical Periodontology* **36**, 661–666.

van der Mei, H. C., White, D. J., Atema-Smith, J., van de Belt-Gritter, E. & Busscher, H. J. (2006) A method to study sustained antimicrobial activity of rinse and dentifrice components on biofilm viability *in vivo*. *Journal of Clinical Periodontology* **33**, 14–20.

Mishima, E. & Sharma, A. (2011) *Tannerella forsythia* invasion in oral epithelial cells requires phosphoinositide 3-kinase activation and clathrin-mediated endocytosis. *Microbiology* **157**, 2382–2391.

Miyazaki, A., Yamaguchi, T., Nishikata, J., Okuda, K., Suda, S., Orima, K., Kobayashi, T., Yamazaki, K., Yoshikawa, E. & Yoshie, H. (2003) Effects of Nd:YAG and CO2 laser treatment and ultrasonic scaling on periodontal pockets of chronic periodontitis subjects. *Journal of Periodontology* **2**, 175–180.

Mombelli, A., Cionca, N. & Almaghlouth, A. (2011) Does adjunctive antimicrobial therapy reduce the perceived need for periodontal surgery? *Periodontology* **2000**, 205–216.

Mombelli, A., Schmid, B., Rutar, A. & Lang, N. P. (2000) Persistence patterns of *Porphyromonas gingivalis, Prevotella intermedia/nigrescens*, and *Actinobacillus actinomyetemcomitans* after mechanical therapy of periodontal disease. *Journal of Periodontology* **71**, 14–21.

Moritz, A., Schoop, U., Goharkhay, K., Schauer, P., Doertbudak, O., Wernisch, J. & Sperr, W. (1998) Treatment of periodontal pockets with a diode laser. *Lasers in Surgery and Medicine* **22**, 302–311.

Nair, S. P., Meghji, S., Wilson, M., Reddi, K., White, P. & Henderson, B. (1996) Bacterially induced bone destruction: mechanisms and misconceptions. *Infection and Immunity* **64**, 2371–2380.

Nomura, K., Yamaguchi, M. & Abiko, Y. (2001) Inhibition of interleukin-1beta production and gene expression in human gingival fibroblasts by low-energy laser irradiation. *Lasers in Medical Science* **16**, 218–223.

Nussbaum, G. & Shapira, L. (2011) How has neutrophil research improved our understanding of periodontal pathogenesis? *Journal of Clinical Periodontology* **38**(Suppl. 11), 49–59.

de Paula Eduardo, C., de Freitas, P. M., Esteves-Oliveira, M., Aranha, A. C., Ramalho, K. M., Simões, A., Bello-Silva, M. S. & Tunér, J. (2010) Laser phototherapy in the treatment of periodontal disease. *Lasers in Medical Science* **25**, 781–792.

Polansky, R., Haas, M., Heschl, A. & Wimmer, G. (2009) Clinical effectiveness of photodynamic therapy in the treatment of periodontitis. *Journal of Clinical Periodontology* **36**, 575–580.

Ready, D., Roberts, A. P., Pratten, J., Spratt, D. A., Wilson, M. & Mullany, P. (2002) Composition and antibiotic resistance profile of microcosm dental plaques before and after exposure to tetracycline. *Journal of Antimicrobial Chemotherapy* **49**, 769–775.

Renvert, S., Lessem, J., Dahlén, G., Lindahl, C. & Svensson, M. (2006) Topical minocycline microspheres versus topical chlorhexidine gel as an adjunct to mechanical debridement of incipient peri-implant infections: a randomized clinical trial. *Journal of Clinical Periodontology* **33**, 362–369.

Rudney, J. D., Chen, R. & Sedgewick, G. J. (2001) Intracellular *Actinobacillus actinomycetemcomitans* and *Porphyromonas gingivalis* in buccal epithelial cells collected from human subjects. *Infection and Immuity* **69**, 2700–2707.

Sbordone, L., Ramaglia, L., Gulletta, E. & Iacono, V. (1990) Recolonization of the subgingival microflora after scaling and root planing in human periodontitis. *Journal of Periodontology* **61**, 579–584.

Schwarz, F., Aoki, A., Becker, J. & Sculean, A. (2008) Laser application in non-surgical periodontal therapy: a systematic review. *Journal of Clinical Periodontology* **35**, 29–44.

Schwarz, F., Sculean, A., Berakdar, M., Georg, T., Reich, E. & Becker, J. (2003a) Clinical evaluation of an Er:YAG laser combined with scaling and root planing for non-surgical periodontal treatment. A controlled, prospective clinical study. *Journal of Clinical Periodontology* **30**, 26–34.

Schwarz, F., Sculean, A., Berakdar, M., Szathmari, L., Georg, T. & Becker, J. (2003b) *In vivo* and *in vitro* effects of an Er:YAG laser, a GaAlAs diode laser, and scaling and root planing on periodontally diseased root surfaces: a comparative histologic study. *Lasers in Surgery and Medicine* **32**, 359–366.

Tester, A. M., Cox, J. H., Connor, A. R., Starr, A. E., Dean, R. A., Puente, X. S., Lopez-Otin, C. & Overal, C. M. (2007) LPS responsiveness and neutrophil chemotaxis *in vivo* require PMN MMP-8 activity. *PLoS One* **2**, e312.

Tomás, I., García-Caballero, L., Cousido, M. C., Limeres, J., Alvarez, M. & Diz, P. (2009) Evaluation of chlorhexidine substantivity on salivary flora by epifluorescence microscopy. *Oral Diseases* **15**, 428–433.

Tribble, G. D. & Lamont, R. J. (2010) Bacterial invasion of epithelial cells and spreading in periodontal tissue. *Periodontology* **2000**, 68–83.

Visser, M. B. & Ellen, R. P. (2011) New insights into the emerging role of oral *spirochaetes* in periodontal disease. *Clinical Microbiology and Infection* **17**, 502–512.

Yilmaz, S., Kuru, B., Kuru, L., Noyan, U., Argun, D. & Kadir, T. (2002) Effect of gallium arsenide diode laser on human periodontal disease: a microbiological and clinical study. *Lasers in Surgery and Medicine* **30**, 60–66.

Address:
Daniele Bani
Section of Histology, Department of Anatomy, Histology & Forensic Medicine
University of Florence
viale G.Pieraccini 6, I-50139, Florence
Italy
E-mail: daniele.bani@unifi.it

Clinical Relevance

Scientific rationale for the study: The use of diode laser in photoablative and photodynamic modes has been proposed as an adjunct to conventional non-surgical therapy for periodontal disease.

Principal findings: The combination of diode laser in photoablative and photodynamic modes with conventional SRP led to significant improvements in clinical parameters (PD, CAL and BOP) and exerted stronger antimicrobial and anti-inflammatory effects to periodontal tissue after a 12-month follow-up than SRP alone.

Practical implications: The present study indicates that diode laser treatment in photoablative and multiple photodynamic modes as adjunct to SRP is a valuable therapeutic option for chronic periodontitis.