

An innovative approach for the treatment of oral diseases

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Abstract

Aim: The purpose of the present study was to evaluate whether irradiation with low-energy Neodymium-doped:yttrium, aluminum, and garnet (Nd:YAG) laser may be a valuable therapeutic approach for the treatment of periodontal and peri-implant diseases. In particular, it was assessed whether laser therapy Nd:YAG is capable to exert a bactericidal effect on microorganisms usually involved in the pathogenesis of periodontal and peri-implant diseases.

Methods: *Actinobacillus actinomycetemcomitans* (*Haemophilus*), aerofil bacterium commonly associated with the peri-implantitis and *Escherichia coli*, used as a model of aerobic Gram-negative bacterium were cultured to assess the bactericidal effect of irradiation with Nd:YAG laser. In some experiments, aliquots (40 µl) of *E. coli* bacterial suspensions were absorbed on the surface of the titanium discs and then exposed to laser treatment.

Results: Laser treatment caused almost a total ablation of the microbial component on the implant surface of titanium without altering the morphological properties. Moreover, the bactericidal effect of the laser also occurred on bacterial cultures in suspension suggesting the effectiveness of this treatment as antiseptic therapy of periodontal pockets and the peri-implant tissues.

Conclusion: Laser therapy, when used with proper working parameters, was able to achieve a consistent microbial ablation of both aerobic and anaerobic species, without damaging the titanium surface.

Keywords: laser therapy, pathogenesis, periodontal, peri-implant.

Introduction

Inflammatory diseases of the tissues are among the most common oral pathologies supporting teeth and dental implants such as periodontal and peri-implant diseases; the latter being the main cause of tooth loss and implant failure in industrialized countries (1). Epidemiological studies have shown that 80% of people suffer from gingival problems with various symptoms such as bleeding, mobility of the teeth, and the like. The periodontal diseases are multi-factorial, but the most important determinant is the colonization of periodontal tissue by different types of germs, particularly *Porphyromonas gingivalis* and *Actinobacillus actinomycetemcomitans* (2). In recent years, the attention of many researchers has been focused on a potential link between periodontal disease and cardiovascular disease (atherosclerosis, ischemic heart disease), (1,3-5) or osteo-arthritis (6). Despite various treatments used to cure periodontal and peri-implant diseases, the incidence of these diseases is still too high in industrialized countries. Conventional treatments consist mainly of procedures involving the mechanical removal of bacterial plaque from tooth surfaces and the use of pharmacological agents such as antibiotics or chemotherapeutics (7-12). However, these conventional treatments have a number of restrictions associated with bacterial resistance, inaccessibility of the instrument to penetrate the dental structures (13-15) and, in particular, some of the recommended methods have been reported to modify and even damage the morphological properties of implant surfaces (16-18). Based on these considerations, it is necessary to find new therapeutic strategies for the treatment of periodontal and peri-implant diseases. In the last decade, the excellent effects of the laser light in cleaning different implant surfaces have been widely reported (19). Despite its anti-microbial property, concerns have been raised against the use of laser treatment, especially in view of the high energy required for the bactericidal effect and the potential heat development. It is likely that the

operating mode may represent an important parameter to be considered for obtaining a successful decontamination and re-osseointegration of the irradiated implants.

The purpose of this study was to assess whether irradiation with a pulsed Nd:YAG laser commonly used in the dental practice is capable, through the selection of the appropriate laser parameters (i.e., low pulse energy) of achieving microbial ablation from titanium without damaging the implant surface properties. In parallel, the bactericidal efficacy of Nd:YAG irradiation at low pulse energy was assessed on bacteria cultured in broth media following exposure to photosensitizing dyes to assess the potential use of this treatment in eliminating bacteria from peri-implant soft tissue.

Methods

Bacterial cultures

For the purpose of this study, two strains of bacteria were used: *Actinobacillus actinomycetemcomitans* (*Haemophilus*), aerofil bacterium, commonly associated with peri-implant diseases, and *Escherichia coli*, used as Gram-negative aerobic model (20,21). The two strains were purchased from ATCC, Manassas, VA, USA. For this purpose, the bacterial suspensions were grown in nutrient agar and resuspended in Luria's Bertani liquid medium (*E. coli*) and in BHI broth (OXOID) supplemented with 5% Fields Enrichment (*A. actinomycetemcomitans*), incubated overnight at 37° C under agitation before irradiation. Bacterial suspensions (40 ml) from each sample were aliquoted in micro-plates and irradiated, colored or not previously with a photosensitizing agent, Methylene Blue 0.02% in water for 2 minutes (Sigma). Samples that were not radiated were used as controls. Each experiment was performed in triplicate. After irradiation, both treated and control samples were diluted 1:1000 in fresh liquidin micro-plates. Colony forming units (CFU) were counted after incubation for 24 h (*E. coli*) and 48h (*A. actinomycetemcomitans*) at 37° C. In some experiments, aliquots (40 µm) of *E. coli*

bacterial suspensions were absorbed on the surface of the titanium disks. The contaminated implants were exposed to laser stimulation for about 3-4 seconds. The surface of the implants exposed to the laser were observed by atomic force microscopy (AFM) after being fixed in alcohol and acetone mixture (1:1) and dried in air. During the laser treatment, in order to minimize the temperature rise of the implant surface, the implants were cooled using air flow. The temperature of the dental implant was monitored thanks to a thermocouple placed on the implant.

Laser irradiation

The irradiation was performed using a pulsed laser of Nd:YAG (Pulse Master 600 IQ, American Dental Technologies Inc., Corpus Christi, TX, USA), acting on a wavelength of 1064nm for a period of 100 microseconds. The diskettes of titanium and biological samples were subjected to irradiation for 3-4 seconds, by means of an optical fiberglass diameter of 400 μm kept at a distance of about 1-2 mm from the sample. The parameters of energy and frequency used are shown in Table 1. To prevent the decrease of efficiency of the laser, the fiber tip was cut after each irradiation.

Atomic force microscopy (AFM)

For this study, an atomic force microscopy (AFM) with a mechanics heads designed and built at the National Institute of Applied Optics (INOA, Italy), was utilized. The head is driven by a mechanical analog control system (SP Magic, by Elbitech Ltd, Marciana, Livorno, Italy). The scanning procedure was a "contact mode" using a triangular cantilever (Microlever by Veeco Metrology Group, Santa

Barbara, CA, USA). For each irradiated area of the disc of titanium, maps of 512x512 pixels with a lateral size of between 10x10 μm (20 nm lateral resolution) and 80x80 pM (160 nm lateral resolution) were obtained.

Assessment of bacterial growth

The bacterial growth was evaluated by counting the colonies that were formed in solid growth medium (Colony Forming Units, CFU/ml). 10 μl of the bacterial culture in liquid medium were plated in a solid growth medium. The plates were placed at 37° C and after incubation, 24 hours (*Escherichia coli*) and 48 hours (*Actinobacillus actinomyces-temcomitans*), the number of colonies formed was evaluated. The growth of bacteria - not subjected to laser treatment (control) - was also evaluated. The experiments were performed in triplicate.

Results

Bactericidal effect of low-energy laser, Nd:YAG

In order to assess the bactericidal effect of laser radiation for a potential use of this treatment in the therapy of periodontal and peri-implant diseases, bacterial cultures were irradiated with laser Nd:YAG, both in suspension and adherent on the titanium discs. In particular, the cultures were irradiated by fixing the parameters of the laser at different energies and frequencies (Table 1) with the aim to identify the conditions of laser treatment that, in parallel with antibacterial effect, does not change the morphology of the implant surfaces. The parameters which best satisfy those needs consist of the following: energy of 20 mJ and a frequency of 50 or 70 Hz (Table 1).

Table 1. Nd:YAG laser parameters

Energy (mJ)	Frequency (Hz)	Power (W)	Comments
100	10	1	alterations
20	50	1	
20	70	1.4	
60	10	0.6	alterations

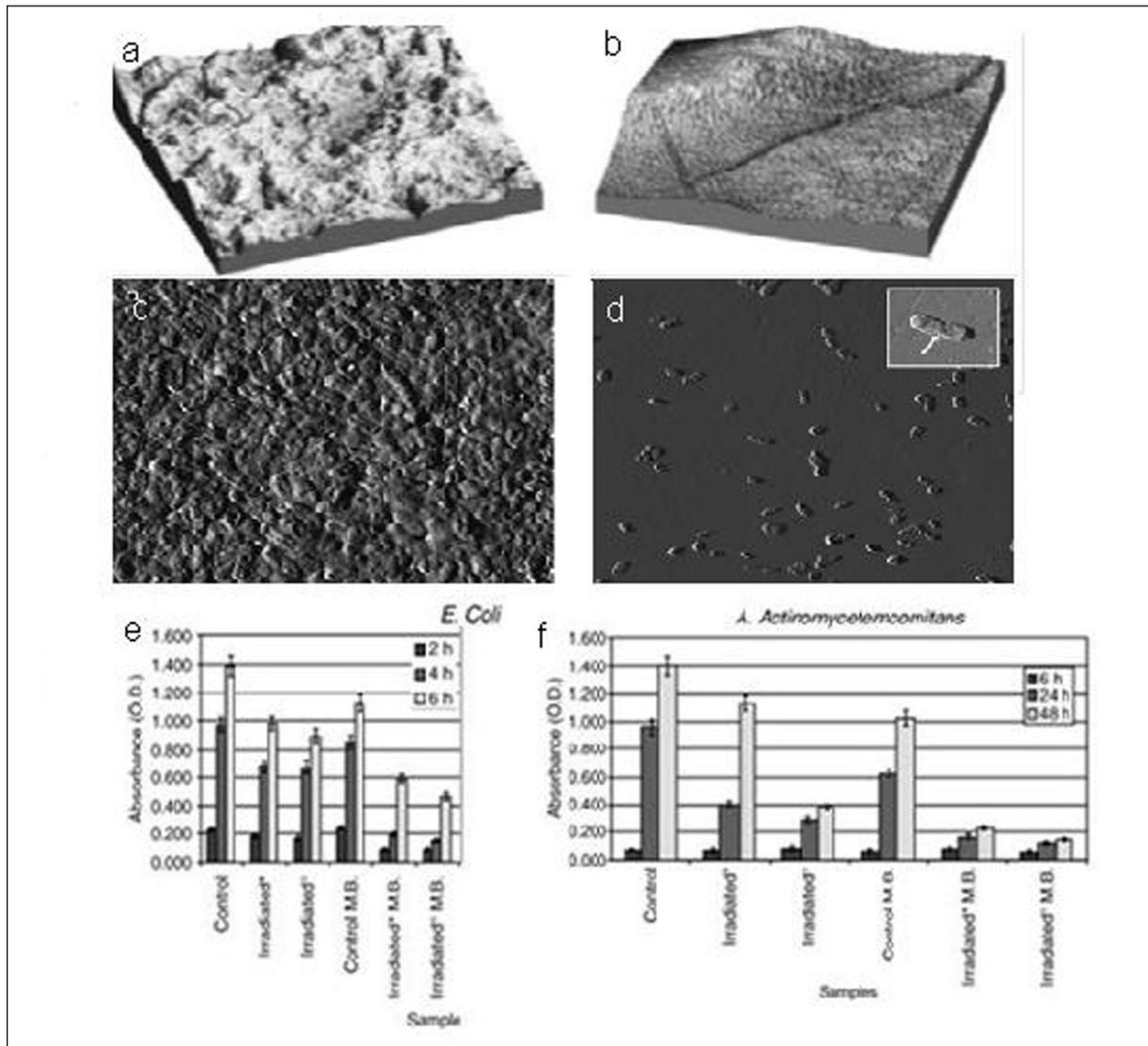
The analysis by atomic force microscopy (AFM) of the surface of titanium implants has allowed the reconstruction of detailed topographic maps of the implant surface of titanium. From these it appeared that the surface roughness (2-3 μM) of dental implants had remained practically unchanged after irradiation at low energy (20 mJ) (Figure 1a), while it was significantly reduced after treatment with the highest energy (100 and 60 mJ) (Figure 1b). Moreover, in addition to preserving the morphological characteristics of the implant, the irradiation with the Nd:YAG laser with energy of 20mJ and frequency of 50-70Hz had a bactericidal effect on microorganisms such as *E. coli* and *A. actinomycetemcomitans* (adsorbed on the surface of the dental implant (Figure 1c, 1d). AFM analysis showed that after treatment with low energy laser, an almost complete removal of the microbial component on the surface of titanium is observed. In particular, on the implants irradiated it was possible to identify only a reduced number of bacteria often with evident breakage of the cell wall. Next, was evaluated the bactericidal effect of low-energy laser on bacterial cultures in suspension in order to verify its antiseptic effectiveness in treating periodontal pockets and peri-implant tissues. For this purpose, bacterial suspensions of *E. coli* and *A. actinomycetemcomitans* were irradiated with the Nd:YAG laser using the same parameters of energy (20 mJ) and frequency (50 Hz to 70 Hz) already adopted for the treatment of titanium implants. After treatment, the suspensions were analyzed to assess bacterial viability. The growth of both bacterial strains, as well as the number of CFU / ml on solid medium, was significantly reduced in the irradiated samples compared to controls (Figure 1e, 1f). Moreover, pretreatment with photosensitive dyes such as Methylene Blue in conjunction with the laser irradiation considerably enhanced the bactericidal efficacy of the treatment with the Nd:YAG laser in the suspension (Figure 1e, 1f).

*Note the absence of alterations and maintenance of surface roughness in the samples irradiated with low-energy laser (20 mJ/50Hz) (a) on contrary the presence of numerous fractures and the reduction of the roughness in the surface of the implants treated with laser to higher energy (100 mJ/10Hz) (b). Representative images by atomic force microscope of *E. coli* bacteria adsorbed on the surface of discs of titanium control (c) and irradiated with Nd:YAG laser 20 mJ/50Hz (d) fixed in alcohol/acetone. Analysis of the growth by counting the colony forming units (CFU) in the solid medium after 24 hours (e), (*E. coli*) and 48 hours (f), (*A. actinomycetemcomitans*) from the seeding of the bacteria control and those undergoing laser treatment in the presence or in the absence of pretreatment with methylene Blue.

Discussion

Nd:YAG laser is one of the most common lasers used for soft tissue dental applications. Several studies recommended the use of these lasers for procedures such as biopsies, phrenectomies, disinfection of the root canal, gingivectomy and gingivoplasty as well as for the treatment of periodontitis and dental abscesses (22-24). On the other hand, information on the potential application of Nd:YAG laser irradiation for the treatment of peri-implantitis and implant failing is limited and somewhat controversial. Indeed, the high bactericidal potential of the Nd:YAG laser irradiation on the titanium implant surfaces has been invariably associated with the occurrence of several side effects including surface alterations and melting of the implants (25-29). In this context, this study suggests that the irradiation of contaminated titanium dental implants, with a pulsed Nd:YAG laser at an ordinary mean power (1–1.4 W), is capable of producing bactericidal ablation without altering the morphological features of the dental implants. In fact, it has been demonstrated that the choice of appropriate laser parameters, namely the laser pulse

Figure 1. Morphological analysis by the atomic force microscope of the surface of titanium implants subjected to laser irradiation (a,b), treatment effect of Nd:YAG laser on the surface of titanium implants contaminated with bacteria (c,d) and analysis of bacterial growth following treatment with Nd:YAG laser combined or not with Methylene Blue (e,f)



energy and repetition rate, is of crucial importance to obtain such beneficial effects on the titanium surfaces. In particular, laser irradiation with a low pulse energy (20 mJ), even at a high repetition rate (50 and 70 Hz), was capable of causing a significant bacterial reduction, without causing undue damage to the titanium implant surface. The bactericidal effects of such irradiation were also examined in *E. coli* and in *A. actinomycetemcomitans* – grown in

suspension and/or sub-cultured on agar medium to examine the potential use of this treatment in tissues, including edema, pus and blood infiltration. Interestingly, it was found that the combination of the laser light with photosensitizing agents was particularly effective in reducing bacterial growth under these experimental conditions.

In conclusion, this study revealed that low-energy laser treatment Nd:YAG can be an effective, safe,

and non-conventional alternative for the treatment of periodontal and peri-implant diseases. Laser therapy was shown to be effective in exerting a selective bactericidal effect on microorganisms, commonly involved on periodontal and peri-implant diseases, without altering the morphology of the

loaded titanium implants.

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Conflict of interest: None declared.

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