Lasers in periodontal therapy

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The term 'LASER' is an acronym of 'Light Amplification by Stimulated Emission of Radiation'. The discovery of lasers was based on the stimulated emission of radiation theory postulated by Einstein. The first laser apparatus was presented by Maiman in 1960 (83). Since then, researchers have explored the effects of low- and high-intensity lasers in dentistry and especially in periodontology.

Phototherapy with low-intensity lasers on wound healing

Treatment with low-intensity lasers to improve wound healing has been applied since 1967, when Mester first showed acceleration of wound healing in mice stimulated with a ruby laser (90). Two theories are accepted to explain the effects of laser irradiation on tissues: the first theory states that the light of a specific wavelength activates the cells' mitochondrial respiratory chain (71); and the second theory assumes that the light acts by opening the calcium channels on the cell membrane (135). Both mechanisms result in an increase of cellular metabolism with a higher production of adenosine triphosphate.

A number of studies have investigated the use of phototherapy to improve wound healing, with conflicting results. Animal studies show either beneficial effects (70, 90, 166) or no effect at all (21, 62, 66, 80, 158). The same applies for human studies, some of which show beneficial effects of laser therapy on wound healing (7, 90), whereas no improvement is reported by others (22, 42, 122). Conversely, cell-culture studies revealed promising results for the action of phototherapy on various biological mechanisms (60, 75, 79, 89, 134).

In our first studies, the effects of phototherapy with a low-intensity laser were investigated on wound healing after gingivoplasty in humans (30, 31). A GaAlAs diode laser with a wavelength of 670 nm (red) and 15-mW power was applied point by point on the mucosa corresponding to the incisors and canines. This study had a split-mouth design and the test side received 4 J/cm² energy density per point, for a total of three points. The application was repeated every 48 h for 1 week (i.e. four sessions in total). Postsurgical clinical evaluation was performed at 7, 15, 21, 30 and 60 days by five blinded experienced periodontists who were asked to choose the side presenting the more advanced gingival healing based on the parameters gingival color, texture and contour. This evaluation resulted in no statistical differences between nontreated sites and sites treated with the low-intensity laser (30). Incisional biopsies taken sequentially at 7, 14, 21 and 60 days postgingivoplasty showed no significant differences in histomorphometric evaluation of the gingival epithelium and connective tissue (31). In contrast to our findings, improved wound healing was found after gingivoplasty in another study using a similar laser-application protocol (7). A visible red laser (685 nm), of 4 J/cm² energy density, was applied by scanning immediately after surgery and 24 h, 3 days and 7 days postoperatively. Clinical measurements of the width of the attached gingiva and the probing depth of the gingival sulcus were made before and immediately after surgery, and at 24 h, and 7, 14, 21, 28 and 35 days postoperatively. At 21 and 28 days the values for probing depth were significantly higher in the control group compared with the laser group ($P < 0.05$), and there were no significant differences in the width of the attached gingiva. Based on these data, the authors assumed that the laser stimulated faster tissue repair compared with no treatment (7).

Challenged by this controversy and aware of the positive effects of laser therapy in cell cultures, we

sought to evaluate the effects of phototherapy at a molecular level. Our research assessed the effects of phototherapy with red (InGaAlP; 660 nm) and infrared (GaAlAs 780 nm) lasers at different energy densities (3 and 5 J/cm^2), but with the same power (40 mW), on the release of growth factors involved in wound healing (29). A primary culture of gingival fibroblasts was treated with two irradiations 6-h apart. The production of basic fibroblast growth factor and keratinocyte growth factor was assessed by ELISA. Similar amounts of keratinocyte growth factor were released following treatment with the different laser wavelengths; however, a significantly greater (1.49 times) amount of basic fibroblast growth factor was released by the cells treated with the infrared laser. This result led us to suggest that one of the mechanisms by which lasers act on wound healing is through an increase in the production of growth factors by cells.

The expression of vascular endothelial growth factor-A was also shown to be enhanced by phototherapy (132). Vascular endothelial growth factor is a key molecule in angiogenesis and vasculogenesis, both of which are important processes in wound healing. Vascular endothelial growth factor is responsible for the proliferation and differentiation of endothelial cells and therefore for the growth of new blood vessels.

There are many variations of parameters among published studies, as presented in Table 1. The biostimulation of phototherapy is dependent on laser-irradiation parameters such as wavelength, laser output power and energy density. These parameters effectively improve cell growth without impairing protein synthesis (103), but it is possible that a parameter able to induce some cells to divide may lead others to apoptose (86). Therefore, it is critical to use the correct combination of parameters to achieve the desired effects.

A study by Azevedo et al. (14) demonstrated that the growth of cultured human fibroblasts can be influenced by the power density of a red laser (GaAlAs 660 nm). There was an inverse relationship between power density and cell growth. Therefore, the lowest power-density tested (10 mW or 142.85 mW/cm^2) elicited the highest cell growth. Another parameter important for affecting cell behavior is the frequency of irradiation. Research on an epithelial Vero cell line (36) demonstrated that cell growth was proportional to the number of irradiations. GaAlAs (660 nm; 40 mW) and InGaAlP (780 nm; 70 mW) lasers were applied, one to three times, at an energy density of 3 or 5 J/cm^2.

Although the triple application improved cell growth, it was insufficient to stimulate the full cell growth observed under regular nutritional conditions.

Phototherapy can exert diverse effects in the same cell line. On osteoblast-like cells phototherapy may act as a proliferative stimulus (50). Fujihara et al. (50) observed that when a GaAlAs laser (780 nm; 40 mW) was applied on osteoblasts (Osteo-1 lineage) in the presence or absence of dexamethasone, the proliferation rate of cells increased independently of the presence of dexamethasone, but the synthesis of osteonectin was not influenced. It was suggested that phototherapy with an infrared laser may act as an important co-adjutant in the acceleration of bone regeneration.

The effect of low-intensity lasers on mast-cell degranulation was shown in two studies (125, 133). The first of these studies had a split-mouth design (133) and used fragments of human gingiva collected during gingivoplasty after irradiation (8 J/cm^2; 50 mW) with infrared (785 nm) and red (688 nm) lasers. After histological preparation, nondegranulated and degranulated mast cells were counted in five areas in the connective tissue. Both irradiation protocols promoted significantly higher mast-cell degranulation than that seen in nonirradiated tissues. The same results were found in the second study in which a red laser was applied (670 nm; 8.0 J/cm^2) and the tissue was obtained from epulis fissuratum (125). These authors showed a possible effect of phototherapy on inflammation because preformed mediators released after degranulation of mast cells can promote inflammation via different actions.

Another mechanism by which low-intensity laser therapy acts on wound healing is through enhancement of blood supply. An investigation in rats failed to prove, by laser Doppler flowmetry, that a HeNe laser (10 mW; 1 J/cm^2) enhances the blood flow in the skin (96). In contrast to this investigation, an immediately induced arteriolar vasodilatation was reported in rat mesentery by the use of low-intensity laser therapy, and this finding was not associated with an elevation in temperature (82).

In summary, several studies show a positive influence of laser therapy on wound healing. These effects occur through the stimulation of miscellaneous biological mechanisms. At the time of surgery, laser therapy produces an increase in blood flow that results in the recruitment of proinflammatory, anti-inflammatory and growth factors to the wound site. At the initial phase of inflammation, laser therapy can stimulate degranulation of mast cells, unleashing the inflammatory response. Thereafter, phototherapy

Table 1. Diversity of parameters among studies on the effect of low-intensity laser on wound healing

Authors	Laser	Wavelength (nm)	Power (mW)	Energy density (J/cm²)	Time	Target	Repetition	Results
Damante et al. (29)	GaAlAs	780	40	3 and 5	3 and 5 s	Human gingival fibroblasts	Twice 6-h interval	Higher production of basic fibroblast growth factor
Damante et al. (30)	GaAlAs	670	15	4	–	Humans	48-h interval for 1 week	No effect on wound healing after gingivoplasty
Amorim et al. (7)	Diode	685	50	4	80 s	Humans	24 h, 3 days, 7 days	Better repair after gingivectomy
Silva et al. (132)	GaAlAs, AlGaInP	780, 660	70, 40	35, 5	40 s, 10 s	Rats	Immediately and 48 h after surgery	Positive influence on vascular endothelial growth factor-A expression
Pereira et al. (103)	GaAs	904	120	3–5	–	NIH3T3 fibroblasts	1–6 days	Stimulation of fibroblast proliferation
Azevedo et al. (14)	GaAlAs	660	10, 29	2	–	Human gingival fibroblasts	Twice, 12-h interval	Lower power stimulates better cell growth
Eduardo et al. (36)	GaAlAs, InGaAlP	660, 780	40, 70	3 or 5	2.8 s, 3.8 s, 1.9 s, 2.5 s	Vero epithelial cells	1, 2 or 3 times	Increased cell growth proportional to number of irradiations
Fujihara et al. (50)	GaAlAs	780	10	3	12 s	Rat osteoblast-like cells	–	Increased cell proliferation
Silveira et al. (133)	GaAlAs	785, 688	50	8	36 s	Human mast cells	–	Promotes cell degranulation
Sawazaki et al. (125)	GaAlAs	670	5	8	4 min	Human mast cells	–	Increased cell degranulation
Núñez et al. (96)	HeNe	–	10	1	3 min	Rats	–	No effects on skin blood flow
Maegawa et al. (82)	GaAlAs	830	30	–	3 min	Rat vascular smooth cells	–	Increased arteriolar blood flow

enhances the proliferation of fibroblasts, osteoblasts and epithelial cells. It also increases protein synthesis and the release of growth factors by these cells. Altogether, these events culminate in faster clinical wound healing.

Lasers for root biomodification

After the first application of a high-intensity laser in dentistry by Fine et al. in 1964 (44), its use was abandoned because the high energy produced excessively elevated temperatures (145). Currently, there are much more controlled devices, including water refrigeration on the Er:YAG laser, which allows the safe use of lasers on hard tissues.

The Er:YAG laser was presented by Zharikov et al. in 1975 (170). Its wavelength of 2940 nm coincides with the absorption peak of water. In the apatite component, hydroxyl radicals also show a relatively high absorption at 2940 nm. As the Er:YAG laser is well absorbed by all biological tissues that contain water, this laser is suitable for the treatment of both soft tissues and hard tissues. The Nd:YAG laser was established by Geusic et al. in 1964 (55), and was the first laser developed exclusively for dentistry (27). It emits a wavelength of 1064 nm and operates on a pulsed mode, with a pulse duration of 150 μs, causing less thermal damage than rubi laser. Its wavelength is absorbed by cellular elements, mainly those containing pigments such as hemoglobin and melanin. It is not well absorbed by hard tissues, in which it causes a melting effect and recrystallization of dentin.

Studies have shown positive effects of lasers on root surfaces, such as the establishment of a biocompatible environment that enhances cell proliferation when dental roots are exposed to laser irradiation. The morphological aspects of sound human-root surfaces irradiated with an Er:YAG laser (47 mJ and 83 mJ) after scaling and root planing were shown by Theodoro et al. (150), who observed that this laser promoted an irregular aspect with efficient removal of the smear layer and exposure of dentinal tubules in the absence of fissures, cracks or carbonized areas. Years later, the same research group (149) compared the same, previously used laser protocol, with citric acid, ethylenediaminetetraacetic acid (EDTA) and citric acid associated with tetracycline, and found no significant differences in smear-layer removal among treatments, but laser irradiation produced a more irregular surface than the etching modalities of root biomodification. Concerning the Nd:YAG laser, Hamaoka et al. (63) applied 2.0 W, 20 Hz, 100 mJ and

124.34 J/cm^2 on the root surfaces of freshly extracted teeth and found fusion and resolidification of the surfaces covering the dentinal tubules. Weaker inflammatory responses and improved biocompatibility were observed for irradiated root fragments implanted in the subcutaneous tissues of rats compared with nonirradiated root fragments.

Human gingival fibroblasts were cultured on dental root fragments after calculus removal followed by Er:YAG irradiation at two different energy densities (60 and 100 mJ/pulse). The best cell adhesion and proliferation was obtained with a pulse of 60 mJ. This energy density creates a homogenous roughness similar to micro-excavations of the same depth and is distributed uniformly along the fragments (38). The adhesion of blood components was also seen on roots modified by Er:YAG (7.6 and 12.9 J/cm^2) and diode (90 and 108 J/cm^2) lasers, forming a dense fibrin network with blood cells attached to it (147). Both energy densities of the Er:YAG laser were more effective compared with the diode laser, which resulted in inhibition and little adhesion of blood components to the surfaces. This can be explained by the poor absorption of the diode laser by water or hydroxyapatite and because its energy is converted to heat. When compared with the control group (scaling and root planing), none of the treatments had statistically significant differences concerning adhesion of blood components (147).

The biocompatibility of periodontally compromised dental roots treated by the Er:YAG laser has been demonstrated in animal research performed by our group (49). Before implantation in rat subcutaneous tissue, root fragments obtained from human teeth extracted because of severely advanced periodontitis were scaled and planed and then treated with the Er:YAG (60 mJ and 100 mJ, 15 s) laser or citric acid. The control group received only scaling and root planing with curettes. Histological analysis after 7, 14 and 28 days identified collagen fibers adhering to root surfaces showing excellent biocompatibility in all experimental groups (Figs 1A–C and 2A–C), except for the control. The differences were statistically significant in relation to the control group, which showed a higher number of inflammatory cells (Fig. 1D) at 7 days and a fibrous capsule surrounding the root fragments at 28 days (Fig. 2D). These results confirm that the Er:YAG laser can promote a biocompatible root surface without a significant inflammatory response.

All the studies discussed had similar results, as shown in the summary presented in Table 2. Based
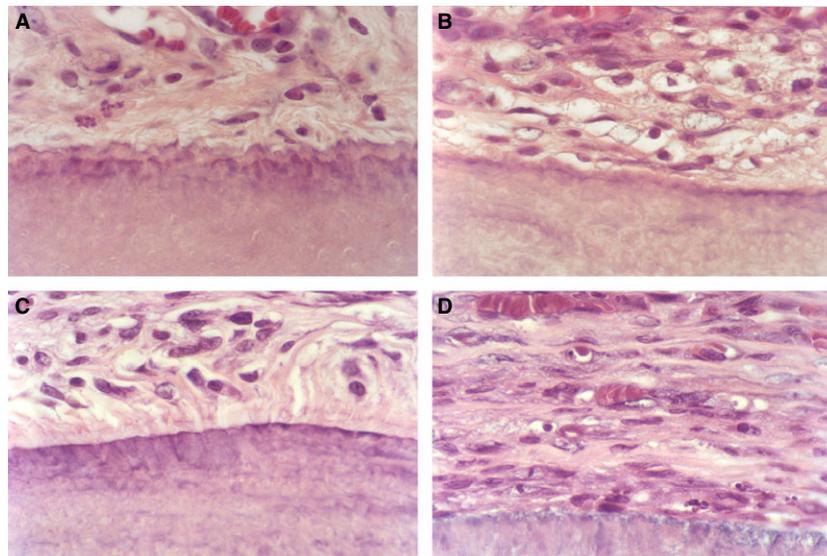
Fig. 1. Histological aspects of tissue adjacent to the root surface after 7 days of implantation. (A) Er:YAG (60 mJ,15 s): high organization pattern of collagen fibers near the irregular root surface. (B) Er:YAG (100 mJ,15 s): presence of fusiform cells resembling fibroblasts. Recently formed collagen fibers with no definition of orientation are present. (C) Citric acid: the collagen fibers are oriented perpendicular to the root surface. (D) Scaling and root planing: collagen fibers are parallel to the root surface. High numbers of inflammatory cells and blood vessels are present (hematoxylin and eosin stain,1000×).
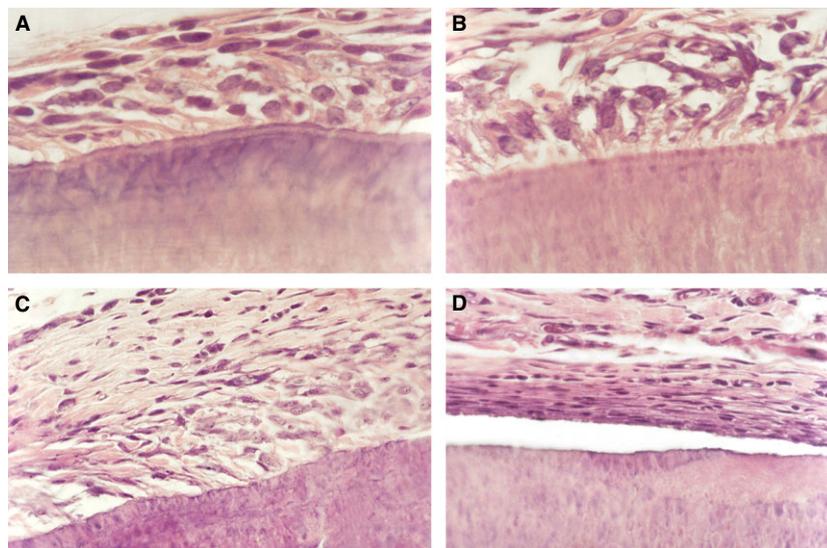

Fig. 2. Histological aspects of tissue adjacent to the root surface after 28 days of implantation. (A) Er:YAG (60 mJ, 15 s): the collagen fibers are oriented oblique to the root surface and suggest adhesion to the surface. (B) Er:YAG (100 mJ, 15 s): the collagen fibers are perpendicular to the root surface. (C) Citric acid: the collagen fibers are oriented oblique to the root surface and suggest adhesion to the surface. (D) Scaling and root planing: there is no interaction of connective tissue with the root surface. Fibrous tissue resembling a capsule with fibers and cells parallel to the surface is present (hematoxylin and eosin stain, 1000× (A, B) and 400× (C, D)).

on this review, it can be assumed that the Er:YAG laser is a good alternative to root biomodification because the irregular surface frequently produced by the Er:YAG laser promotes only a weak initial inflammatory response while efficiently removing the smear layer, and enhances growth and adhesion of cells as well as blood components.

Lasers as an adjunct tool for treatment of periodontal disease

As stated above, lasers are capable of creating a biocompatible root surface favorable for periodontal healing, as attested by animal and human studies.

Table 2. Studies on root biomodification with high-intensity lasers

Authors	Laser	Wavelength	Power (W)	Frequency (Hz)	Energy density/ pulse (mJ)	Energy density (J/cm²)	Time (s)	Target	Results
Theodoro et al. (150)	Er:YAG	2.94 μm	–	10	47 and 83	0.57 and 1.03	15	Root fragments	Irregular surface with no fissure, cracks or carbonized area
Franco et al. (49)	Er:YAG	2.94 μm	–	10	60 and 100	–	15	Root fragments Implantation on rat subcutaneous tissue	More biocompatibility and similar results to citric acid
Feist et al. (38)	Er:YAG	2.94 μm	–	10	60 and 100	3 and 5	10	Root surfaces Human gingival fibroblasts	60 mJ – faster cell adhesion and growth
Theodoro et al. (147)	Er:YAG and GaAlAs	2.94 μm and 808 nm	– 0.9, 1.08	10	60, 100	7.6, 12.0 and 90, 108	15	Root fragments Blood components	No differences to control. Er:YAG more adhesion of blood components
Hamaoka et al. (63)	Nd:YAG	1.064 μm	2	20	100	124.34	10	Root surfaces Implantation on ra subcutaneous tissue	Improved biocompatibility
Theodoro et al. (149)	Er:YAG	2.94 μm	–	10	47 and 83	5.8 and 10.3	15	Root fragments	Significant differences from control. Similar to citric acid, EDTA, citric acid + tetracycline

Andrade et al. (9) evaluated reduction of bacteria, after Nd:YAG laser irradiation, in class II furcation defects in patients with chronic periodontitis. The laser (100 mJ/pulse, 1.5 W, 15 Hz, duration of 150 μs and energy density of 141.5 J/cm^2) was applied after scaling and root planing. The treatment was repeated after 1 week. There were no differences between groups in relation to improvement of clinical parameters, but a significant reduction in the number of colony-forming units of total bacteria was found, mainly for the experimental group immediately after treatment. The number of dark-pigmented bacteria returned to the initial counts after 6 weeks. This study shows that the Nd:YAG laser is a promising instrument for reducing the number of bacteria in periodontal pockets.

Another study on bacterial reduction was performed in rats by Fontana et al. (47). After inducing periodontal disease by ligature, periodontal pockets were irradiated with an 819-nm diode laser with power of 400, 600 and 800 mW, and 1 and 1.2 W, for 9 s. Laser treatment had bactericidal and fungicidal effects, with the highest reductions observed for *Prevotella* sp. and *Fusobacterium* sp., even without scaling and root planing before laser irradiation. Another interesting finding was that the laser parameters used did not induce a rise in temperature capable of causing irreversible damage to the periodontal tissues (48).

The low-intensity laser, as an adjunct to the treatment of periodontal defects, was tested in rats in a study showing the positive effect of an 830-nm laser (40 mW, 4 J/cm^2) applied at four points around the osseous defects filled with inorganic bovine bone (108). The results showed increased repair, as judged by both bone formation and the amount of collagen fibers surrounding the graft, for laser-treated defects compared with nonirradiated defects. Similar effects were observed with the addition of a collagen membrane covering the bone defects (54). Furthermore, a positive biomodulative effect was found in bone healing at sites grafted with autologous bone, either alone (160) or combined with bone morphogenetic proteins plus irradiation of the surgical bed before grafting (151). Similar results were obtained recently by Pinheiro et al. (109) following the use of a low-intensity laser (850 nm, 150 mW, 4 J/cm^2), at 48-h intervals for 15 days combined with a mineral trioxide aggregate graft and membrane in osseous defects in rats. They concluded that phototherapy improved the results of the use of mineral trioxide aggregate, showing marked deposition of new bone.

Low-intensity laser, in conjunction with scaling and root planing, may be a valuable tool in immunosuppressed individuals, as attested by a study in rats (52). The rats received injections of dexamethasone, and then periodontal disease was induced with cotton ligatures. The experimental group received scaling and root planing plus irradiation with a diode (GaAlAs; 660 nm) laser in three points of application on each tooth with 30 mW power and 57.14 J/cm^2 energy density per point for 133 s. A radiographic and histological analysis showed significantly less bone loss in the experimental groups. This research reinforces an important finding that immunosuppressed subjects can exhibit improved wound healing in response to phototherapy.

Laser in dentin hypersensitivity

Dentin hypersensitivity is a common occurrence in sites of gingival recession caused by exposure of dentinal tubules to the oral environment (45). The pain generated ranges from mild to severe after physical (cold, heat) and/or mechanical (mainly toothbrushing trauma) stimuli (16, 45, 126) at the exposed area, but it can also be triggered by chemical or osmotic stimuli (17, 26). The average prevalence of dental hypersensitivity varies from 8.9% to 15% in the Western adult population (126), but there are reports computing a prevalence of 74% of the total population (17) and of up to 98% of patients after periodontal therapy (26). Some explanations for this discrepancy are the large variations in the methods of data collection and in the inclusion criteria. Periodontally compromised individuals, the presence of gingival recession and smokers show a higher incidence of dental hypersensitivity (17). The most affected teeth are premolars, followed by first molars and incisors (17). A slightly higher incidence of tooth sensitivity has been reported in women than in men (17).

Literature reports indicate that the majority of patients with dental hypersensitivity are 20–40 years of age (17, 76) with the peak incidence occurring at the end of the third decade of life, and diminishing during the fourth and fifth decades. This can be explained by the decrease in dentin permeability and in neural sensitivity with age as a response to the natural deposition of secondary sclerotic dentin and also as a result of the prolonged use of fluoride dentifrices (17).

Changes in lifestyle and diet may be related to increased loss of the hard substrate as a result of corrosive processes. The frequent consumption of bever-

ages with demineralizing properties, and nutrition disorders such as bulimia and gastroesophageal reflux, may contribute to an increased incidence of noncarious lesions associated with dental hypersensitivity (88). It is considered that the hypersensitive dentin is permeable to the movement of fluid within the tubules, which can transport excitatory agents to the pulp nerve endings (10). Therefore, the treatment of dental hypersensitivity is intended not only to restore the original impermeability of dentinal tubules, but also to control the inflammatory changes that generate a pulpal manifestation of pain. Toothpastes and desensitizing substances applied topically are the most popular treatment methods, but their effectiveness is highly variable (16).

The effectiveness of laser in reducing dentin hypersensitivity is also controversial. A recent systematic review (128) aiming to identify randomized, placebo-controlled clinical trials concluded that laser treatment can reduce dental hypersensitivity, but not significantly when compared with placebo treatment. Middle- (Nd Nd:YAG, CO_2 and Er:YAG) and low-level (He-Ne and GaAlAs) output power lasers have been used to treat dental hypersensitivity. Middle-output lasers seal or occlude the dentinal tubules by melting and recrystallizing the dentin (72). Low-level lasers can act directly on the pulp nerve terminals, causing analgesia by depressing the transmission of nerve stimulation, and may occlude dentinal tubules by increasing the cellular metabolic activity of odontoblasts that promote tertiary dentin production (43).

Lasers may enhance the effects of other desensitizing agents. Favorable results were obtained in an *in vitro* study in which the CO_2 laser was irradiated on dentin devoid of cementum after application of a calcium hydroxide paste (120). This study showed higher occlusion of dentinal tubules when the association was used compared with laser alone. The laser produced melting, recrystallization, cracking and carbonization of dentin surfaces, but the temperature rise was below 5°C. The melting and resolidification of dentin in the presence of craters and cracks were also verified by Glauche et al. (57) and Ciaramicoli et al. (25) using an Nd:YAG laser. An evaluation by energy-dispersive X-ray microanalysis showed that the Nd:YAG laser, used in combination with metal salts, produced structural changes in the dentin surface, such that tin, strontium and fluorine were found in depths ranging from 250 to 500 μm (57).

The Er:YAG laser has been considered appropriate in the treatment of dental hypersensitivity and some of its properties may explain why. The Er:YAG laser has a water-absorption characteristic approximately 15 times greater than that of CO_2 and even 20,000 times greater than that of the Nd:YAG laser (159). Therefore, the Er:YAG laser would evaporate the superficial layers of the dentinal fluid, resulting in a decrease of dental hypersensitivity (126). In addition, thermal damage, such as cracking, melting or charring, was not observed with the Er:YAG laser (16).

Perhaps the biggest challenge in the use of lasers in the treatment of dental hypersensitivity is the establishment of parameters for their clinical use. An *in vitro* study of erbium lasers compared the wavelengths of 2.94 μm (Er:YAG laser) and 2.78 μm (Er,Cr: YSGG laser) at defocused mode in different settings and application times (10). The results showed that no parameter was able to completely seal the dentinal tubules, but dental hypersensitivity decreased after irradiation with Er:YAG and Er,Cr:YSGG lasers at the 0.25–0.50 W settings (11).

The low-intensity lasers have also shown variable results. A decrease in dental hypersensitivity was observed in a clinical trial after application of red (660 nm) and infrared (830 nm) lasers for 114 s on hypersensitive teeth (76). The red laser showed a greater degree of desensitization in subjects 25–35 years of age compared with the infrared laser. This age group also showed a higher rate of desensitization compared with subjects 35–45 years of age, who are more prone to regressive or atrophic changes in the dentin–pulp complex resulting from the physiological aging process. In this study, the infrared laser was ineffective in subjects 35–45 years of age. Desensitization was attributed to removal of the nociceptive potential of pulp nerve fibers. The red laser (660 nm) was also compared with the light-emitting diode (630 ± 10 nm) and a placebo in six sessions (78), with similar results among all treatments at 15 days and better results for the laser at 60 days. This study also demonstrated that two sessions seem to be sufficient for reducing dental hypersensitivity. Conversely, a study by our team (53) compared the effect of a light-cured composite resin (placebo) with a GaAlAs diode laser (670 nm) in six applications with a 48- to 72-h interval between applications. After 8 weeks, pain reduction was observed with both treatments without significant differences between them. Using similar methodology, Lizarelli et al. (77) also found no difference between the infrared laser and light-emitting diode, but both produced more reduction in dentin sensitivity compared with placebo. The authors attributed the results to the production of reactionary dentin through a physiological nonaggressive pathway.

Recently, Flecha et al. (45) compared laser irradiation with cyanoacrylate adhesive application in dental hypersensitivity reduction. They employed diode infrared laser (660 nm GaAlAs) or cyanoacrylate glue in three sessions with a 48-h interval between sessions. Both treatments showed significant reduction of hypersensitivity, with no statistical difference between them, up to 6 months of follow-up. It was concluded that cyanoacrylate is safe and as effective as low-intensity laser therapy in reducing hypersensitivity, as well as being more accessible and cheaper than the high-intensity lasers.

Photodynamic therapy

Photodynamic therapy is a treatment modality based on the activation of exogenous photosensitizing agents by a light source to produce cell damage. This action was first observed in 1900 by Raab (116), who realized that a protozoon could be killed in the presence of acridine excited by a visible light. Photosensitizing agents or photosensitizers are dyes composed of molecules capable of absorbing light energy and using it to promote chemical reactions in cells and tissues when exposed to light (167). In order to have the desired effect, the color of the dye used in photodynamic therapy must be compatible with the wavelength of the light, must have minimal toxicity and must have high absorption at the resonant wavelengths of the more efficient lasers (69). A photosensitizer bonded to bacteria can be activated by light of the appropriate wavelength in the presence of oxygen to generate singlet oxygen and free radicals that are cytotoxic to microorganisms, mainly as a result of damage to the cytoplasmic membrane and DNA (121). This phenomenon is referred to as lethal photosensitization (161) and if the target cells are microorganisms, photodynamic therapy is often referred to as antimicrobial photodynamic therapy (144).

Once a photosensitizer is exposed to a resonant visible light band, it becomes activated to a short-lived singlet state. By losing energy or by means of a physical process, this unstable molecule either returns to the stable state or may form a longer-lived triplet state. At this stage, the molecule can undergo redox reactions with surrounding molecules (reaction type I), or can produce reactive oxygen species such as peroxides, superoxide ions, hydroxyl radicals and singlet oxygen ($^1O_2^*$) (reaction type II) (51, 58, 59, 112). The reactive oxygen species react with cellular components, such as proteins, organelles, nucleic acids and lipids, causing irreversible damage as a result of modification to the respiratory chain (157), increased membrane permeability and cell death (40, 56, 100, 102). An overview of the current status, mechanisms of action, applications and new frontiers of photodynamic therapy was recently published in *Periodontology 2000* by Soukos & Goodson (139).

The use of light with a well-defined wavelength selected to match specifically with the photosensitizer (67, 142, 169) has been recommended, and selection of a photosensitizer, in turn, is essential for the success of photodynamic therapy. The most studied photosensitizers are hematoporphyrin derivatives (620–650 nm), phenothiazine (620–700 nm), cyanine (600–805 nm), phytotherapic agents (550–700 nm), phthalocyanines (660–700 nm), xanthene derivatives, acridines, chlorins and merocyanines (87, 100, 105, 111, 131, 157). Phenothiazine dyes have intense absorption in the region of 620–660 nm and thus are useful in photodynamic therapy because they are within the therapeutic window required not only for the efficient penetration of light in tissue but also for the sufficient production of singlet oxygen (155). Within the phenothiazine family, toluidine blue O and methylene blue are the most frequently used photosensitizers. Studies have shown that toluidine blue O is effective against various bacteria, including species found in the oral cavity (124). Toluidine blue O interacts with lipopolysaccharides present in the cell membrane of gram-negative bacteria, even in the absence of light (152) but, when exposed to a wavelength of 630 nm, it has maximal absorption and good photodynamic properties for killing various microbes *in vitro* (169). Methylene blue, in turn, shows maximal absorbance when exposed to a wavelength of 660 nm (24).

Toluidine blue O is preferred as a photosensitizer by some researchers (5, 40, 136, 137) because, owing to its hydrophilicity and low molecular weight, it can pass easily through the cell membrane (68). These properties are shared by methylene blue (156), but toluidine blue O interacts with lipopolysaccharide of gram-negative bacteria better than does methylene blue (152). Nevertheless, discoloration of the dental structure was observed with phenothiazine derivatives (107). Pinheiro et al. (110) used 50% toluidine blue (0.005% mg/500 ml, diluted in 50% Endo-PTC base paste) to minimize dental discoloration. The same approach has been used with azulene in a paste-base delivery system in order to avoid bluish staining on implant surfaces (64).

Goulart et al. (58, 59) demonstrated in 2010, for the first time, that it is possible to generate reactive oxygen by activation of rose bengal, erythrosine and

methylene blue using a conventional dental light-curing unit because the absorption spectrum of these photosensitizing agents is the same as that emitted by the light-curing unit (300–800 nm). This treatment was effective against *Aggregatibacter actinomycetemcomitans* in planktonic and biofilm cultures, with erythrosine being more effective than methylene blue (59). Erythrosine was also found to have great potential in the treatment of oral biofilms (163). The ideal concentration of rose bengal was determined as up to 0.1 μM (maximal absorption peak at 560 nm) during 1 min of irradiation, and a 55% reduction of *A. actinomycetemcomitans* viability was obtained in planktonic cultures. The reduction in biofilm (of about 45%) was significantly dependent on the concentration of rose bengal and the irradiation time, and no effect on gingival fibroblasts was observed (58).

Malachite green had never been used as a photosensitizer in antimicrobial photodynamic therapy until Prates et al. (114) investigated its ability to kill *A. actinomycetemcomitans* when combined with a low-power red laser (GaAlAs; 660 nm). A reduction, of up to 99%, in the number of colony-forming units of *A. actinomycetemcomitans* was observed following exposure to malachite green (0.01%, weight per volume) for 5 min and irradiation for 3 or 5 min.

The biological response to photodynamic therapy may be influenced by the concentration of dye, the period of pre-irradiation, the pH of the environment, the presence of exudates, the light source, the energy dose and the fluence rate applied (28, 162). Regarding the pre-irradiation time, researchers (3, 4, 40, 41, 98, 113) generally advocate that 1 min is sufficient to achieve cellular uptake of toluidine blue O, and 5 min is sufficient to achieve cellular uptake of methylene blue (46, 93) and other blue and green dyes (114, 127). A dose-dependent relationship seems to exist between the concentration of toluidine blue O and its lethal effect, that is, the greater the concentration of dye, the lower the number of microorganisms (81). By contrast, Soares et al. (136) found that low concentrations of toluidine blue O, of 50 μM and 25 μM, produced optimal killing of *Cryptococus gattii*, and this finding might be a result of the observation that antimicrobial photodynamic therapy should be less efficient at higher concentrations of toluidine blue O because the photosensitizer target becomes saturated, leaving a substantial pool of unbound toluidine blue O that can absorb photons of light away from toluidine blue O associated with cells (67). Some studies also found that an increase in methylene blue concentration caused a decrease in the number of colony-forming units recovered after irradiation (56, 58).

The effects of antimicrobial photodynamic therapy on biofilms, periodontal diseases and peri-implantitis, in the view of Latin-American authors, are presented below and a summary of the main results is given in Table 3.

Effects of antimicrobial photodynamic therapy on biofilms

Although antibiotics have been considered to eliminate residual periodontal pathogens after scaling and root planing (118), studies have shown that an organized biofilm exhibits several resistance mechanisms (8, 33) that protect periodontal pathogens and limit the action of the antibiotics (32). Moreover, some treatment strategies for periodontitis, using tetracycline and metronidazole, have shown that it is difficult to maintain therapeutic concentrations of these agents in the periodontal pocket for a sufficient length of time to ensure total elimination of bacteria (68, 87, 162). Additionally, some drawbacks have been reported in the application of local or systemic antibiotics, such as gastrointestinal disorders, problems with patient compliance (101) and development of bacterial resistance to the antibiotics (39). Therefore, antimicrobial photodynamic therapy started to be used instead of topical antibiotics because a wide variety of microorganisms in the oral cavity can be killed by antimicrobial photodynamic therapy (73, 93, 162). However, when organized in biofilms, oral bacteria are less affected by antimicrobial photodynamic therapy compared with bacteria in planktonic cultures. Fontana et al. (46) found that approximately 63% of bacteria from human dental plaque in the planktonic phase were killed, compared with the maximal killing of 32% of bacteria in biofilms, following treatment with methylene blue (25 μg/ml) for 5 min and exposure to red light (665 nm). In a different protocol, methylene blue (0.1 mg/ml for 5 min of pre-irradiation) and InGaAlP laser (660 nm for 98 s) (104) produced significant decreases in the viability of biofilms formed by *Candida albicans, Staphylococcus aureus* and *Streptococcus mutans* grown in acrylic discs, predominantly in the outermost layers of the biofilms. However, the reduction in the number of colony-forming units produced by the photodynamic therapy was more significant in single-species biofilms than in biofilms composed by association with different microbial species.

Recently, a study from our team evaluated the effect of antimicrobial photodynamic therapy on biofilms grown on sand-blasted, large grit, acid etched

Table 3. Effects of antimicrobial photodynamic therapy on biofilms, periodontal diseases and peri-implantitis

Study type/Authors	Light source	Protocol	Target	Outcomes
Antimicrobial photodynamic therapy on biofilms				
Goulart et al. (58,59)	Conventional light-curing unit for composite resin (400–500 nm)	Rose bengal, erythrosine and methylene blue, 0.1 μM for 1 min + irradiation for 10 s to 3 min	*Aggregatibacter actinomycetemcomitans* in planktonic and biofilm cultures	55% reduction of *Aggregatibacter actinomycetemcomitans* in planktonic cultures and 45% reduction in biofilms. No effects on fibroblasts
Prates et al. (114)	GaAlAs (660 nm)	Malachite green (0.01% weight per volume) for 5 min + irradiation for 3 or 5 min	*Aggregatibacter actinomycetemcomitans* in cultures	Reduction of up to 99% in the number of colony-forming units
Soares et al. (136)	InGaAlP (630 nm)	Toluidine blue O 27.04 μg/ml, 13.52 μg/ml, 6.76 μg/ml; variable energy, density and irradiation time	*Cryptococcus gattii*	50 μM and 25 μM toluidine blue O produced optimal reduction of *Cryptococcus gattii* viability, including isolates resistant to antifungals
Fontana et al. (46)	Diode laser (665 nm)	Methylene blue (25 μg/ml and 50 μg/ml) for 5 min + irradiation	Suspensions of plaque microorganisms and multispecies biofilms	63% bacterial reduction in plaque suspension and 32% bacterial reduction in biofilms
Pereira et al. (104)	InGaAlP (660 nm)	Methylene blue (0.1 mg/ml) for 5 min + irradiation for 98 s	Biofilms formed *by Candida albicans, Staphylococcus aureus* and *Streptococcus mutans* in acrylic	Significant decreases in the viability of biofilms
Zapata et al. (168)	InGaAlP (660 nm)	Toluidine blue O (100 μg/ml) for 1 min + irradiation for 1 min	Polymicrobial biofilms formed on sand-blasted, large-grit, acid-etched titanium surface	Bacteriostatic effect of antimicrobial photodynamic therapy in the first 24 h + significant reduction in the number of colony-forming units
Antimicrobial photodynamic therapy in periodontal diseases				
Almeida et al. (4, 5)	GaAlAs (685 nm)	Methylene blue (100 mg/ml) for 1 min; low-level laser therapy for 2 min; Methylene blue (100 mg/ml) for 1min + low-level laser therapy for 2 min	Effects on the progression of experimentally induced periodontitis in rats	Less bone loss/less inflammatory reaction of photodynamic therapy compared with no treatment at 5 and 15 days postoperatively. No significant difference among groups at 30 days

Table 3. (*Continued*)

Study type/Authors	Light source	Protocol	Target	Outcomes
Prates et al. (113)	Diode laser (660 nm)	Methylene blue (100 μM) for 5′ + irradiation for 1′; saline control	Effects on the tissues and subgingival bacterial growth in experimentally induced periodontitis in rats	Better periodontal healing, as measured by collagen organization, inflammatory infiltrate and bone loss + higher microbial reduction observed in the photodynamic therapy group
Theodoro et al. (148)	GaAlAs (660 nm)	Scaling and root planing vs. scaling and root planing + toluidine blue O (100 μg/ml) for 1′; scaling and root planing + toluidine blue O (100 μg/ml) for 1′ + irradiation for 150′′	Effects on clinical and microbiological parameters of human sites with chronic periodontitis	At 180 days, antimicrobial photodynamic therapy led to a significant reduction in the percentage of sites positive for *Aggregatibacter actinomycetemcomitans, Porphyromonas gingivalis, Prevotella intermedia, Tannerella forsythia* and *Prevotella nigrescens* compared with scaling and root planing alone, but produced no statistically significant benefits in clinical outcome
Pinheiro et al. (110)	Diode laser (632.8 nm)	Toluidine blue O (0.005 mg/500 ml) + a mixture of urea peroxide, polysorbate at 15% and polyethylene glycol (vehycle) for 3′ + irradiation for 1′; scaling and root planing	Bacteria from periodontal pockets of human chronic periodontitis	Reduction of 81.24% after scaling and root planing and of 95.90% after photodynamic therapy in the number of viable bacteria
Oliveira et al. (100)	Diode laser (660 nm), 60 mW/cm²	Scaling and root planing; phenothiazine chloride (10 mg/ml) for 1′ + irradiation for 1′	Clinical periodontal parameters of human aggressive periodontitis	Photodynamic therapy and scaling and root planing lead to significant improvements in the clinical parameters after 3 months without statistically significant differences between them
Novaes et al. (95)	Diode laser (660 nm), 60 mW/cm²	Scaling and root planing; phenothiazine chloride (10 mg/ml) for 1′ + irradiation for 1′	Human subgingival microbiological composition in aggressive periodontitis	Antimicrobial photodynamic therapy was more effective in reducing the numbers of *Aggregatibacter actinomycetemcomitans* than was scaling and root planing; scaling and root planing was more efficient in reducing pathogens of the red complex

Table 3. (*Continued*)

Study type/Authors	Light source	Protocol	Target	Outcomes
Antimicrobial photodynamic therapy in peri-implantitis				
Hayek et al. (64)	GaAlAs (660 nm), 40 mW, 7.2 J	25% azulene for 5′ + irradiation for 3′; mucoperiosteal flaps + scaling + chlorhexidine irrigation	Microbial reduction in ligature-induced peri-implantitis in dogs	Significant reduction in *Prevotella* sp., *Fusobacterium* sp. and beta-hemolytic *Streptococcus* sp. immediately after treatment, without significant differences between groups
Shibli et al. (129)	GaAlAs (685 nm), 4 J	Toluidine blue O (100 µg/ml) for 1′ + irradiation for 80″+ expanded polytetrafluoroethylene membrane (guided bone regeneration) (variable types of implant surfaces)	Healing potential after surgical treatment in ligature-induced peri-implantitis in dogs	Re-osseointegration achieved in all types of surfaces, 3 months after surgery; bone fill varied from 48.28% in hydroxylapatite-coated implants to 26.70% in commercially pure titanium implants
Shibli et al. (130)	GaAlAs (830 nm), 50 mW, 4 J	Toluidine blue O (100 µg/ml) for 1′+ irradiation for 80″ + guided bone regeneration; debridement + guided bone regeneration; debridement only	Healing potential after surgical treatment in ligature-induced peri-implantitis in dogs	After 5 months, antimicrobial photodynamic therapy showed significantly higher bone gain, better re-osseointegration and 5.6 times less exposure of membranes than the other treatments, regardless of the implant surface
Salmeron et al. (123)	InGaAlP (660 nm), 30 mW, 45 J/cm^2	Toluidine blue O (100 µg/ml) for 1′ only; toluidine blue O (100 µg/ml) for 1′+ irradiation for 1′; irradiation only	Rats' connective tissue reaction to rough- and smooth-surface implants contaminated with human dental plaque	Less inflammation for antimicrobial photodynamic therapy after 7 days, but over longer periods all methods produced outcomes equivalent to sterile implants

(SLA) titanium surfaces (168). A pool of bacteria from microbial plaque collected from an adult male individual with generalized chronic periodontitis was used to contaminate titanium discs that had been decontaminated by antimicrobial photodynamic therapy (toluidine blue O, 100 μg/ml for 1 min + InGaAlP laser, wavelength = 660 nm, for 1 min) and processed for colony-forming unit counting. A bacteriostatic effect of antimicrobial photodynamic therapy was observed in the first 24 h and the number of colony-forming units was significantly reduced when compared with nondecontaminated discs, although it remained higher than for sterile discs.

Some studies (152, 153) have demonstrated that gram-positive bacteria are susceptible to photodynamic inactivation, but gram-negative bacteria are significantly resistant to many photosensitizers used in antimicrobial photodynamic therapy (85). However, some microbial species, such as the oral black-pigmented bacteria, naturally contain photosensitizers and are very susceptible to antimicrobial photodynamic therapy. Soukos et al. (140) demonstrated that a light band ranging from 380 to 520 nm was able to achieve a threefold reduction in the growth of *Porphyromonas gingivalis*, *Prevotella intermedia*, *Prevotella nigrescens* and *Prevotella melaninogenica* in dental plaque samples obtained from human subjects with chronic periodontitis. Based on these findings, Soukos & Goodson (139) proposed a phototherapeutic strategy by which daily exposure to visible light would gradually suppress the numbers of black-pigmented bacteria, leading to a shift of the microbial environment toward a new one associated with health. It is worth noting the data from a recent review by Vera et al. (154) who found no consensus as to which is the most reliable model for evaluating photodynamic therapy efficacy against biofilms. They observed that the majority of published reports use methodologies in which biofilms are grown on plastic or silicon microtiter plates and surfaces and emphasized that these bioassays have been repetitively criticized for lack of robustness and occasionally yield inconsistent results.

Antimicrobial photodynamic therapy in periodontal disease

It is well accepted that mechanical removal of the oral biofilm with hand instruments is a prerequisite for long-term success in periodontal therapy, resulting in significant clinical improvements in the great majority of cases (15, 92). Nevertheless, scaling and root planing with curettes requires a certain level of skill, is time consuming and may be difficult because of complex root morphologies, as found in furcation areas (117). There is evidence that periodontopathogens, such as *Tannerella forsythia* and *A. actinomycetemcomitans*, remain in periodontal pockets after nonsurgical therapy (119, 143), and bacterial recolonization in the subgingival environment occurs even shortly after scaling and root planing (95, 98), requiring regular supportive periodontal therapy. In the past decade, these limitations of conventional periodontal therapy gave rise to many attempts to introduce antimicrobial photodynamic therapy as an alternative for the adjuvant treatment of chronic periodontitis (3, 4, 20, 51, 68, 87).

Some advantages are frequently cited for antimicrobial photodynamic therapy in relation to other periodontal treatments. The photosensitizer placed directly into the pocket can be activated either through the thin gingival tissues or via an optical fiber placed directly in the pocket, thus avoiding damage to adjacent host tissues (115); the activity of antimicrobial photodynamic therapy is initiated only when exposed to a light source, thus preventing the selection of resistant bacterial species (84); used during the maintenance period of periodontal therapy, it avoids removal of additional root substance by mechanical retreatment and consequent dentin hypersensitivity (87); and the treatment time is reduced because anesthesia is unnecessary and destruction of bacteria occurs within a very short period of time (about 60 s), avoiding damage to the adjacent host tissues (73).

Despite the abundance of promising data on the advantages of its use, there is still controversy regarding the real benefits of antimicrobial photodynamic therapy in periodontal treatment. In 2010, a systematic review and meta-analysis was published to verify the effects of antimicrobial photodynamic therapy on periodontal parameters of adult subjects with periodontitis (13). The review recruited five studies that met the inclusion criteria (randomized controlled trials comparing photodynamic therapy with a placebo, and no intervention or scaling and root planing). Small sample sizes, moderate to high risk of biases and clinical heterogeneities among the included studies, were common shortcomings, and the main conclusion was that antimicrobial photodynamic therapy as an independent treatment or as an adjunct to scaling and root planing did not demonstrate statistically or clinically significant advantages. Therefore, the authors did not recommend routine use of antimicro-

bial photodynamic therapy for clinical management of periodontitis. Curiously, in the same year, another meta-analysis on the same subject (12) reached the opposite conclusions. That review included four studies that met the inclusion criteria (randomized controlled trials comparing scaling and root planing/ antimicrobial photodynamic therapy with scaling and root planing alone), three of which matched the first review. The data were considered as supportive of the potential improvements of antimicrobial photodynamic therapy in conjunction with scaling and root planing in periodontal treatment because this association was significantly related to greater clinical attachment gain and reduction in probing depth.

Indeed, satisfactory results with the use of antimicrobial photodynamic therapy as an adjunct to scaling and root planing have been reported in experimental periodontitis produced in animals (3–5), but clinical studies have often shown no beneficial effects of antimicrobial photodynamic therapy when compared with scaling and root planing alone (139). The progression of experimentally induced periodontal disease was significantly influenced by antimicrobial photodynamic therapy as radiographically and histologically demonstrated by Almeida et al. (4), in rats. In their study, methylene blue (100 mg/ml) followed by GaAlAs laser of 685 nm wavelength produced less bone loss compared with no treatment. Positive results with the same treatment protocol were also found in furcation areas (5) compared with methylene blue alone or no treatment. Better periodontal healing, as measured by collagen organization, inflammatory infiltrate and bone loss for antimicrobial photodynamic therapy compared with areas treated with distilled water, was also reported by Prates et al. (113). These findings were observed when sites of experimentally produced periodontitis in rats were treated with diode laser (660 nm wavelength for 1 min) and methylene blue (100 μM). Additionally, in another group of animals in which the induction of periodontitis was associated with inoculation with *A. actinomycetemcomitans* collected from a patient with aggressive periodontitis, significant bacterial reduction was achieved immediately following both types of treatment, with higher microbial reduction observed for the antimicrobial photodynamic therapy.

Such good results have not always been seen in human studies. Theodoro et al. (148) found no significant difference when comparing the clinical and microbiological effects of scaling and root planing alone, scaling and root planing plus irrigation with toluidine blue O, and an antimicrobial photodynamic therapy using toluidine blue O, in patients with

chronic periodontitis. Clinical parameters, including visible plaque index, gingival index, bleeding on probing, probing depth, gingival recession and clinical attachment level, were all improved from baseline up to 180 days. At 180 days, antimicrobial photodynamic therapy showed a significant reduction in the percentage of sites positive for *A. actinomycetemcomitans*, *P. gingivalis*, *P. intermedia*, *T. forsythia* and *P. nigrescens* compared with scaling and root planing alone, but produced no statistically significant benefits in terms of clinical outcome. Conversely, favorable results were reported in patients with chronic periodontitis by Pinheiro et al. (110), who found reduction of 81.2% and 95.9% in the number of viable bacteria in periodontal pockets, respectively, after scaling and root planing and antimicrobial photodynamic therapy (a mixture of toluidine blue O and Endo-PTC® for 3 min, followed by diode laser (632.8 nm) for 1 min).

A sequence of studies on the effects of antimicrobial photodynamic therapy in the treatment of aggressive periodontitis (95, 99, 100) reported favorable results in reducing the human subgingival microbiota. In one of these studies (95), subgingival plaque samples were collected from patients with aggressive periodontitis and the counts of 40 subgingival species were determined using checkerboard DNA–DNA hybridization. Treating the affected sites with antimicrobial photodynamic therapy (1 min of pre-irradiation with phenothiazine chloride plus diode laser, wavelength = 660 nm, for 1 min) reduced the number of *A. actinomycetemcomitans* better than did scaling and root planing. By contrast, scaling and root planing was more efficient than antimicrobial photodynamic therapy in reducing the presence of periodontal pathogens of the red complex. Nevertheless, recolonization in the sites treated with antimicrobial photodynamic therapy was observed, especially for *T. forsythia* and *P. gingivalis*. As antimicrobial photodynamic therapy and scaling and root planing affected different groups of bacteria, it was suggested that their combined use may be beneficial for the nonsurgical treatment of aggressive periodontitis (95). Previous results from a randomized controlled clinical trial (100) indicated that after 3 months both treatments (antimicrobial photodynamic therapy and scaling and root planing) yielded comparable clinical outcomes in terms of reducing bleeding on probing, reducing probing pocket depths and gaining clinical attachment levels.

The microbiological profile and cytokine pattern were affected by antimicrobial photodynamic therapy in ligature-induced periodontal disease in dogs in a

study by Oliveira et al. (98). One week after a single application of antimicrobial photodynamic therapy alone (pre-irradiation for 1 min with phenothiazine chloride plus 660 nm diode laser for 1 min), scaling and root planing alone or the combination of both treatments, all resulted in reduction in the levels of most bacterial species. However, an increase in the counts of *P. intermedia*, *P. nigrescens* and *T. forsythia* was observed for antimicrobial photodynamic therapy, alone, and in conjunction with scaling and root planing. After 4 weeks, regrowth of *P. gingivalis* and *Treponema denticola* was observed for all treatments and a remarkable reduction of counts for *A. actinomycetemcomitans* was observed for the antimicrobial photodynamic therapy. Additionally, a reduction in the expression of cytokines and bacterial load was observed regardless of the treatment used. The authors inferred that the different periodontal treatments tested present distinct mechanisms of action on the microbiota and thus might have additive, or even synergistic, effects.

Effects of photodynamic therapy on cells and tissues

A possible concern regarding photodynamic therapy would be the potential photocytotoxicity to human cells. However, it has been demonstrated that the doses of light required to kill bacteria in photodynamic therapy are far lower than the one that is toxic to human keratinocytes and fibroblasts (141). Indeed, some beneficial effects of photodynamic therapy have been reported in periodontal ligament cells, such as inhibition of the production of inflammatory mediators, thus favoring cellular chemotaxis and the promotion of local vasodilation and angiogenesis (65).

It has been demonstrated that photodynamic therapy can influence the behavior of inflammatory cells during human chronic periodontitis. Antigen-presenting cells (macrophages and Langerhans' cells) are particularly sensitive to photodynamic therapy and hence their numbers, or their capacity to activate T-lymphocytes, can be reduced (127), thereby diminishing the inflammatory response. Séguier et al. (127) found that photodynamic therapy targeted different cell populations depending on the drug-delivery systems used as photosensitizers (liposomes or nanoemulsions). Thus, the authors observed that nanoemulsions could lead to the migration of Langerhans' cells toward the gingival connective tissue for antigen presentation; liposomes, in turn, reduced the

number of macrophages and increased the density of the gingival collagen. These events are beneficial to the area affected by inflammation as macrophages phagocyte collagen fibers and produce proteases and cytokines that participate in periodontal tissue destruction.

Antimicrobial photodynamic therapy in peri-implantitis

Peri-implantitis was defined by Albrektsson & Isidor (2) as an inflammatory process that affects the hard and soft tissues around implants in function, resulting in bone loss. The development of peri-implantitis requires prior colonization of the implant surface in the form of bacterial biofilm (91). Decontamination by mechanical, chemical and physical methods has been used in the treatment of peri-implantitis. However, rough implant surfaces may be very difficult to decontaminate as bacteria are protected in microirregularities or undercuts of the surface (91). Antimicrobial photodynamic therapy has been extensively studied regarding its effectiveness for cleaning different implant surfaces (35, 61, 64, 123, 168), but so far complete decontamination has not been achieved.

Plaque-induced peri-implantitis in dogs treated with antimicrobial photodynamic therapy using 25% azulene in a paste-base delivery (left in place for 5 min) and the GaAlAs low-power laser (wavelength = 660 nm for 3 min) resulted in a significant reduction in the numbers of *Prevotella* sp., *Fusobacterium* sp. and beta-hemolytic *Streptococcus* sp. immediately after treatment (64). However, the reduction in microbes was not significantly different when antimicrobial photodynamic therapy was compared with conventional flap therapy and irrigation with chlorhexidine.

A study conducted in humans by Dörtbudak et al. (35) revealed more favorable results. Their experiment, involving photosensitization with toluidine blue O plus irradiation with a diode laser (wavelength = 690 nm) for 1 min, resulted in significant bacterial reduction on implant surfaces showing clinical and radiographic signs of peri-implantitis. Interestingly, the authors observed that the application of toluidine blue O alone resulted in significant reductions of *P. intermedia* and *A. actinomycetemcomitans*, but not of *P. gingivalis*, compared with the initial bacterial counts. This finding could not be attributed to a greater susceptibility of black-pigmented bacteria to the dye, but it was suggested that a variable bonding behavior of the dye to different bacterial membranes may occur. The photosensitization provided by toluidine blue O caused

damage to the bacterial membrane when the dye was activated by the laser. When studying methods for the treatment of peri-implantitis, the authors focused on the potential of these methods to convert previously contaminated metal surfaces into surfaces capable of re-osseointegration. It was concluded that lethal photosensitization may be useful as an adjunct to surgical debridement and guided bone regeneration.

The results of experimental peri-implantitis in which contaminated implants of different surface roughness were treated with antimicrobial photodynamic therapy (toluidine blue O, 100 mg/ml + Ga-AlAs, wavelength = 830nm) showed significantly higher bone gain, better re-osseointegration and 5.6 times less exposure of membranes compared with implants treated with surgical debridement only or guided bone regeneration only, regardless of the type of implant surface (129, 130).

A study conducted by our group (123) investigated the intensity of the inflammatory infiltrate and the degree of fibrosis produced in rat subcutaneous connective tissue following the implantation of contaminated titanium discs after decontamination with toluidine blue O, low-intensity laser therapy or antimicrobial photodynamic therapy plus toluidine blue O. It was verified that antimicrobial photodynamic therapy produced the best outcome compared with the other methods after 7 days, but over longer periods all methods produced outcomes equivalent to sterile implants. This information is especially important when considering a protocol for treating peri-implantitis because in the first days of the tissue-repair process, cells coming from the epithelial, connective and bone tissue 'compete' for colonization of the surgical wound. In clinical conditions, epithelial cells would be the first to reach the metallic implant surface over a period of 7–15 days (97). It seems that decontamination methods need to show differences during this period if they are to be considered as useful in treating peri-implantitis. If the behavior shown by antimicrobial photodynamic therapy in this study can be duplicated in the actual peri-implant environment, it is possible that bone precursor cells may be capable of adhering to treated surfaces from which epithelial cells are excluded, thereby favoring re-osseointegration. The fact that all groups showed a tissue reaction equivalent to sterile implants after 28 days raises the possibility that contaminated implants (even untreated ones) placed in living tissue with no access to the external environment may eventually be well tolerated by the body because the hermetic environment of living tissue enables the host immune mechanisms, after a certain time, to eliminate the causative agent of the inflammatory reaction (123).

It is worth noting that the use of antimicrobial photodynamic therapy to decontaminate implant surfaces or in periodontics has an important limitation when applied in the clinical setting, namely the presence of bleeding. Excessive bleeding in the area to be treated can interfere with the effectiveness of photodynamic therapy because the blood absorbs much of the energy of lasers, which therefore cannot penetrate into deep areas of a periodontal defect, for example (34, 35). Furthermore, being red, the blood can absorb a large portion of energy produced by light located out of the red band (<620 nm or >740 nm approximately). In the case of a red laser, blood would not compete with blue dyes but it could act as a physical barrier, thus preventing the dye reaching the bacterial membrane (74). If this is the case, good control of trans-surgical bleeding during antimicrobial photodynamic therapy is recommended.

Specific indications for antimicrobial photodynamic therapy in periodontics

There is increasing evidence that antimicrobial photodynamic therapy can be a useful tool for antibacterial treatment of specific periodontal conditions when conventional therapy with hand instruments is not effective (3), in medically compromised individuals (3, 94, 138), in children and in disabled people (164). It has also been suggested that antimicrobial photodynamic therapy could be justified as an adjunct to conventional periodontal treatment in persistent periodontitis that is strongly related to the presence of *P. gingivalis* and *P. intermedia* (1).

The use of antimicrobial photodynamic therapy has counteracted the impaired healing frequently seen in subjects with diabetes and/or immunosuppressed individuals (18, 106, 146). In immunosuppressed rats, it was observed that the clinical signs of gingival inflammation were exaggerated; there was less adhesion of gingival tissue to the tooth, greater bone loss in furcation areas and a more disorganized tissue space, with a discrete number of fibroblasts, compared with immunocompetent animals (19). However, antimicrobial photodynamic therapy has been proven to reduce bone loss and to promote the repair of bone tissue previously altered by high doses of immunosuppressive drugs (19, 40, 41, 52). The beneficial effect of photodynamic therapy in immunosuppressed individuals has been attributed to the

photodestructive effects on the different reactive oxygen species responsible for irreversible damage to the bacterial cytoplasmic membrane, including protein modification, respiratory chain breakdown and nucleic acid alterations, and also to the increased angiogenesis that brings more oxygen to the area (24, 106).

Periodontal disease in individuals with AIDS is a frequent occurrence (37) and is usually treated by scaling and root planing plus antimicrobials (165). The use of antimicrobial photodynamic therapy as an adjunctive therapy in HIV-associated periodontitis was first reported in 2012 by Noro Filho et al. (94). The authors treated 12 patients with HIV, either with scaling and root planing alone or with scaling and root planing plus antimicrobial photodynamic therapy (methylene blue for 5 min followed by GaAlAs, 660 nm for 133 s), and observed that patients treated with scaling and root planing plus antimicrobial photodynamic therapy showed greater periodontal probing depth reduction and clinical attachment level gain compared with those treated with scaling and root planing only, up to 6 months after treatment. Microbiologically, both therapies presented a reduction in the numbers of *P. gingivalis*, *T. forsythia* and *A. actinomycetemcomitans* detected, without significant differences between them. The results led the authors to imply that HIV-associated periodontitis could be one of the main diseases targeted with applications of photodynamic therapy because patients with HIV are recognized immunosuppressed individuals and are more susceptible to opportunistic infections.

The influence of antimicrobial photodynamic therapy on periodontal bone loss related to diabetes induced in rats was first reported by Almeida et al., in 2008 (3), whose data histometrically demonstrated that antimicrobial photodynamic therapy (using toluidine blue O at 100 mg/ml and a GaAlAs laser) produced less bone loss in both diabetic and nondiabetic rats compared with rats treated only with scaling and root planing, toluidine blue O or low-intensity laser therapy. The authors considered that antimicrobial photodynamic therapy compensated for the lack of bone formation of the alveolar bone in the diabetic rats by increasing the diffusion of oxygen through the tissue, favoring the repair process, as collagen secretion by fibroblasts in the extracellular space occurs only in the presence of high rates of oxygen pressure. Conversely, antimicrobial photodynamic therapy showed no benefit when compared with conventional nonsurgical periodontal therapy in a clinical survey on 45 patient with diabetes in whom scaling and root planing, scaling and root planing plus systemic doxycycline, and scaling and root planing plus antimicrobial photodynamic therapy resulted in the absence of significant differences in plaque and bleeding scores, probing depth, clinical attachment level and glycosylated hemoglobin levels, 3 months after treatment (6).

Conclusions

Latin-American authors have spent considerable efforts to elucidate the biological effects of high- and low-intensity lasers, used alone or in association with photosensitizing agents. Although the use of lasers in periodontics and dental implants has demonstrated promising outcomes *in vitro*, the results are still conflicting and difficult to extrapolate to clinical practice. Induction of growth factors is one of the cellular effects produced by laser irradiation that explains the acceleration of wound healing. The effects on root dentin, either in the removal of smear layer or in dentin desensitization, are still highly varied and controversial. When used in antimicrobial photodynamic therapy, lasers reduce the presence and action of bacteria in dental biofilms and in biofilms formed on the metallic surfaces of implants, favoring regenerative procedures. However, the wide variety of protocols makes comparisons among studies very difficult. As the biological effects are highly dependent on the wavelength and the irradiation parameters of the laser used, it becomes imperative to search for an application pattern that achieves consistency between clinical and laboratory use. Moreover, it is necessary that, once established, this pattern proves advantageous as an alternative or adjunct to conventional treatment. Consistent use of lasers in periodontics seems to find scientific support in individuals with systemic alterations that compromise the immune system or in those unable to undergo invasive treatments. In these individuals, the biostimulating effects of irradiation, associated or not with photosensitizers, seem to counteract the cellular adverse effects produced by the disease.

Acknowledgements

References

1. Aimetti M, Romano F, Torta I, Caposio P, Romagnoli R. Debridement and local application of tetracycline-loaded fibres in the management of persistent periodontitis: results after 12 months. *J Clin Periodontol* 2004: **31**: 166–172.

2. Albrektsson T, Isidor F. Consensus report of Session IV. In: Lang P, Karring T, editors. *Proceedings of the 1st European workshop on periodontology*. London: Quintessence Publishing Co. Ltda.,1994: 365–369.

3. Almeida JM, Theodoro LH, Bosco AF, Nagata MJ, Bonfante S, Garcia VG. Treatment of experimental periodontal disease by photodynamic therapy in rats with diabetes. *J Periodontol* 2008: **79**: 2156–2165.

4. Almeida JM, Theodoro LH, Bosco AF, Nagata MJ, Oshiiwa M, Garcia VG. Influence of photodynamic therapy on the development of ligature-induced periodontitis in rats. *J Periodontol* 2007: **78**: 566–575.

5. Almeida JM, Theodoro LH, Bosco AF, Nagata MJH, Oshiiwa M, Garcia VG. In vivo effect of photodynamic therapy on periodontal bone loss in dental furcations. *J Periodontol* 2008: **79**: 1081–1088.

6. Al-Zahrani MS, Bamshmous SO, Alhassani AA, Al-Sherbini MM. Short-term effects of photodynamic therapy on periodontal status and glycemic control of patients with diabetes. *J Periodontol* 2009: **80**: 1568–1573.

7. Amorim JCF, Sousa GR, Silveira LB, Prates RA, Pinotti M, Ribeiro MS. Clinical study of the gingiva healing after gingivectomy and low-level laser therapy. *Photomed Laser Surg* 2006: **24**: 588–594.

8. Anderson GG, O'Toole GA. Innate and induced resistance mechanisms of bacterial biofilms. *Curr Top Microbiol Immunol* 2008: **322**: 85–105.

9. Andrade AKP, Feist IS, Pannuti CM, Cai S, Zezell DM, De Micheli G. Nd:YAG laser clinical assisted in class II furcation treatment. *Lasers Med Sci* 2008: **23**: 341–347.

10. Aranha ACC, Eduardo CP. In vitro effects of Er,Cr:YSGG laser on dentine hypersensivity. Dentine permeability and scanning electron microscopy analysis. *Lasers Med Sci* 2012: **27**: 827–834.

11. Aranha ACC, Eduardo CP. Effects of Er:YAG and Er,Cr:YSGG laser on dentine hypersensivity. Short-term clinical evaluation. *Lasers Med Sci* 2012: **27**: 813–818.

12. Atieh MA. Photodynamic therapy as an adjunctive treatment for chronic periodontitis: a meta-analysis. *Lasers Med Sci* 2010: **25**: 605–613.

13. Azarpazhooh A, Shah PS, Tenenbaum HC, Goldberg MB. The effect of photodynamic therapy for periodontitis: a systematic review and meta-analysis. *J Periodontol* 2010: **81**: 4–14.

14. Azevedo LH, Eduardo FP, Moreira MS, Eduardo CP, Marques MM. Influence of different power densities of LILT on cultured human fibroblast growth. A pilot study. *Lasers Med Sci* 2006: **21**: 86–89.

15. Badersten A, Nilvéus R, Egelberg J. 4-year observations of basic periodontal therapy. *J Clin Periodontol* 1987: **14**: 438–444.

16. Badran Z, Boutigny H, Struillou X, Baroth S, Laboux O, Soueidan A. Tooth desensitization with an Er:YAG laser: in vitro micrsocopical observation and a case report. *Lasers Med Sci* 2011: **26**: 139–142.

17. Bartold PM. Dentinal hypersensitivity: a review. *Aust Dent J* 2006: **51**: 212–218.

18. Bondan EF, Lallo MA, Baz EI, Sinhorini IL, Graça DL. Ultrastructural study of the remyelinating process following local ethidium bromide injection in the brainstem of dexamethasone-immunosuppressed rats. *Arq Neuropsiquiatr* 2004: **62**: 131–138.

19. Bottura PE, Milanezi J, Fernandes LA, Caldas HC, Abbud-Filho M, Garcia VG, Baptista MA. Nonsurgical periodontal therapy combined with laser and photodynamic therapies for periodontal disease in immunosuppressed rats. *Transplant Proc* 2011: **43**: 2009–2016.

20. Braun A, Dehn C, Krause F, Jepsen S. Short-term clinical effects of adjunctive antimicrobial photodynamic therapy in periodontal treatment: a randomized clinical trial. *J Clin Periodontol* 2008: **35**: 877–884.

21. Braverman B, McCarthy RJ, Ivankovich AD, Forde DE, Overfield M, Bapna MS. Effect of helium-neon and infrared laser irradiation on wound healing in rabbits. *Lasers Surg Med* 1989: **9**: 50–58.

22. Carrillo JS, Calatayud J, Manso FJ, Barberia E, Martinez JM, Donado M. A randomized double blind clinical trial on the effectiveness of helium-neon laser in the prevention of pain, swelling and trismus after removal of impacted third molars. *Int Dent J* 1990: **40**: 31–36.

23. Cavagni J, Soletti AC, Gaio EJ, Rosing CK. The effect of dexamethasone in the pathogenesis of ligature-induced periodontal disease in Wistar rats. *Braz Oral Res* 2005: **19**: 290–294.

24. Chan Y, Lai CH. Bactericidal effects of different laser wavelengths on periodontopathic germs in photodynamic therapy. *Lasers Med Sci* 2003: **18**: 51–55.

25. Ciaramicoli MT, Carvalho RCR, Eduardo CP. Treatment of cervical dentin hypersensivity using neodymium:ytrium-aluminium-garnet laser. Clinical evaluation. *Lasers Surg Med* 2003: **33**: 358–362.

26. Clavijo EMA, Clavijo VRG, Bandéca MC, Nadalin MR, Andrade MF, Saad JRC, Pelegrine AA. Clinical efficiency of low-level diode laser in reducing dentin hypersensivity. *Laser Phys* 2009: **19**: 2041–2044.

27. Coluzzi DJ. An overview of laser wavelengths used in dentistry. *Dent Clin North Am* 2000: **44**: 753–765.

28. Costa L, Carvalho CM, Faustino MA, Neves MG, Tomé JP, Tomé AC, Cavaleiro JA, Cunha A, Almeida A. Sewage bacteriophage inactivation by cationic porphyrins: influence of light parameters. *Photochem Photobiol Sci* 2010: **9**: 1126–1133.

29. Damante CA, De Micheli G, Miyagi SPH, Feist IS, Marques MM. Effect of laser phototherapy on the release of fibroblast growth factors by human gingival fibroblasts. *Lasers Med Sci* 2009: **24**: 885–891.

30. Damante CA, Greghi SLA, Sant'Ana ACP, Passanezi E, Taga R. Histomorphometric study of the healing of human oral mucosa after gingivoplasty and low-level laser therapy. *Lasers Surg Med* 2004: **35**: 377–384.

31. Damante CA, Greghi SLA, Sant'Ana ACP, Passanezi E. Clinical evaluation of the effects of low intensity laser (GaAlAs) on wound healing after gingivoplasty in humans. *J Appl Oral Sci* 2004: **12**: 133–136.

32. Darveau RP, Tanner A, Page RC. The microbial challenge in periodontitis. *Periodontol 2000* 1997: **14**: 12–32.

33. del Pozo JL, Patel R. The challenge of treating biofilm associated bacterial infections. *Clin Pharmacol Ther* 2007: **82**: 204–209.

34. Deppe H, Horch HH, Neff A. Conventional versus CO2 laser-assisted treatment of peri-implant defects with the concomitant use of pure-phase β-tricalcium phosphate: a 5-year clinical report. *Int J Oral Maxillofac Implants* 2007: **22**: 79–86.

35. Dörtbudak O, Haas R, Bernhart T, Mailath-Pokorny G. Lethal photosensitization for decontamination of implant surfaces in the treatment of peri-implantitis. *Clin Oral Implants Res* 2001: **12**: 104–108.

36. Eduardo FP, Mehnert DU, Monezi TA, Zezell DM, Schubert MM, Eduardo CP, Marques MM. Cultured epithelial cells response to phototherapy with low intensity laser. *Lasers Surg Med* 2007: **39**: 365–372.

37. Engeland CG, Jang P, Alves M, Marucha PT, Califano J. HIV infection and tooth loss. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 2008: **105**: 321–326.

38. Feist IS, De Micheli G, Carneiro SRS, Eduardo CP, Miyagi SPH, Marques MM. Adhesion and growth of cultured human gingival fibroblasts on periodontally involved root surfaces treated by Er:YAG laser. *J Periodontol* 2003: **74**: 1368–1375.

39. Feres M, Haffajee AD, Allard K, Som S, Goodson JM, Socransky SS. Antibiotic resistance of subgingival species during and after antibiotic therapy. *J Clin Periodontol* 2002: **29**: 724–735.

40. Fernandes LA, de Almeida JM, Theodoro LH, Bosco AF, Nagata MJ, Martins TM, Okamoto T, Garcia VG. Treatment of experimental periodontal disease by photodynamic therapy in immunossupressed rats. *J Clin Periodontol* 2009: **36**: 219–228.

41. Fernandes LA, Martins TM, de Almeida JM, Theodoro LH, Garcia VG. Radiographic assessment of photodynamic therapy as an adjunctive treatment on induced periodontitis in immunosuppressed rats. *J Appl Oral Sci* 2010: **18**: 237–243.

42. Fernando S, Hill CM, Walker R. A randomized double blind comparative study of low level laser therapy following surgical extraction of lower third molar teeth. *Br J Oral Maxillofac Surg* 1993: **31**: 170–172.

43. Ferreira AN, Silveira L, Genovese WJ, de Araújo VC, Frigo L, de Mesquita RA, Guedes E. Effect of GaAlAs laser on reactional dentinogenesis induction in human teeth. *Photomed Laser Surg* 2006: **24**: 358–365.

44. Fine S, Maiman TH, Klein E, Scott RE. Biological effects of high peak power radiation. *Life Sci* 1964: **3**: 209–222.

45. Flecha OD, Azevedo CGS, Matos FR, Barbosa NMV, Ramos-Jorge ML, Gonçalves PF, Silva EMK. Cyanoacrylate versus laser in the treatment of dentin hypersensitivity: controlled, randomized, double-blind and non-inferiority clinical trial. *J Periodontol* 2013: **84**: 287–294.

46. Fontana CR, Abernethy AD, Som S, Ruggiero K, Doucette S, Marcantonio RC, Boussios CI, Kent R, Goodson JM, Tanner AC, Soukos NS. The antibacterial effect of photodynamic therapy in dental plaque derived biofilms. *J Periodontal Res* 2009: **44**: 751–759.

47. Fontana CR, Kurachi C, Mendonça CR, Bagnato VS. Microbial reduction in periodontal pockets under exposition of a medium power diode laser: an experimental study in rats. *Lasers Surg Med* 2004: **35**: 263–268.

48. Fontana CR, Kurachi C, Mendonça CR, Bagnato VS. Temperature variation at soft periodontal and rat bone tissues during a medium-power diode laser exposure. *Photomed Laser Surg* 2004: **22**: 519–522.

49. Franco EJ, Greghi SLA, Assis GF. Comparative evaluation of radicular periodontal therapies with conventional and Er: YAG lasers as from the rats' subcutaneous tissue reaction. *Rev Intern Period Clínica* 2004: **1**: 60–65 (Portuguese).

50. Fujihara NA, Hiraki KRN, Marques MM. Irradiation at 780 nm increases proliferation rate of osteoblasts independently of dexamethasone presence. *Lasers Surg Med* 2006: **38**: 332–336.

51. Garcez AS, Nunez SC, Hamblim MR, Suzuki H, Ribeiro MS. Photodynamic therapy associated with conventional endodontic treatment in patients with antibiotic-resistant microflora: a preliminary report. *J Endod* 2010: **36**: 1463–1466.

52. Garcia VG, Fernandes LA, de Almeida JM, Bosco AF, Nagata MJ, Martins TM, Okamoto T, Theodoro LH. Comparison between laser therapy and non-surgical therapy for periodontitis in rats treated with dexamethasone. *Lasers Med Sci* 2010: **25**: 197–206.

53. Gentile LCG, Greghi SLA. Clinical evaluation of dentin hypersensivity treatment with the low intensity Gallium-Aluminium-Arsenide laser – AsGaAl. *J Appl Oral Sci* 2004: **12**: 267–272.

54. Gerbi MEM, Pinheiro ALB, Marzola C, Limeira Junior FA, Ramalho LMP, Ponzi EAC, Soares AO, Carvalho LCB, Lima HVL, Gonçalves TO. Assessment of bone repair associated with the use of organic bovine bone and membrane irradiated at 830 nm. *Photomed Laser Surg* 2005: **23**: 382–388.

55. Geusic JE, Marcos HW, Van Uitert LLG. Laser oscillations in Nd-doped yttrium aluminum, yttrium gallium, and gadolinium garnets. *Appl Phys Lett* 1964: **4**: 182–184.

56. Giroldo LM, Felipe MP, Oliveira MA, Munin E, Alves LP, Costa MS. Photodynamic antimicrobial chemotherapy (PACT) with methylene blue increases membrane permeability in *Candida albicans*. *Lasers Med Sci* 2009: **24**: 109–112.

57. Glauche CE, Freitas PM, Vieira N Jr, Marques JLL. Qualitative microanalysis of ions and ultrastructural changes in dentin exposed to laser irradiation and to metal salts solution. *Lasers Surg Med* 2005: **36**: 334–339.

58. Goulart RC, Thedei G Jr, Souza SL, Tedesco AC, Ciancaglini P. Comparative study of methylene blue and erythrosine dyes employed in photodynamic therapy for inactivation of planktonic and biofilm-cultivated *Aggregatibacter actinomycetemcomitans*. *Photomed Laser Surg* 2010: **28**: 85–90.

59. Goulart R, de C, Bolean M, Paulino T, de P, Thedei Jr G, Souza SL, Tedesco AC, Ciancaglini P. Photodynamic therapy in planktonic and biofilm cultures of *Aggregatibacter actinomycetemcomitans*. *Photomed. Laser Surg* 2010: **28**: 53–60.

60. Grossman N. 780 nm low power diode laser irradiation stimulates proliferation of keratinocyte cultures: involvement of reactive oxygen species. *Lasers Surg Med* 1998: **22**: 212–218.

61. Haas R, Dörtbudak O, Mensdorff-Pouilly N, Mailath G. Elimination of bacteria on different implant surfaces through photosensitization and soft laser. An in vitro study. *Clin Oral Implants Res* 1997: **8**: 249–254.

62. Hall G, Anneroth G, Schennings T, Zetterqvist L, Rydén H. Effect of low-level energy laser irradiation on wound healing. An experimental study in rats. *Swed Dent J* 1994: **18**: 29–34.

63. Hamaoka L, Moura-Netto C, Marques MM, de Moura AA. Nd:YAG laser improves biocompatibility of human dental root surfaces. *Photomed Laser Surg* 2009: **27**: 715–720.

64. Hayek RR, Araújo NS, Gioso MA, Ferreira J, Baptista-Sobrinho CA, Yamada AM, Ribeiro MS. Comparative study between the effects of photodynamic therapy and conventional therapy on microbial reduction in ligature-induced peri-implantitis in dogs. *J Periodontol* 2005: **76**: 1275–1281.

65. Houreld N, Abrahamse H. In vitro exposure of wounded diabetic fibroblast cells to a helium-neon laser at 5 and 16 J/cm2. *Photomed Laser Surg* 2007: **25**: 78–84.

66. In de Braekt MM, vanAlphen FA, Kuijpers-Jagtman AM, Maltha JC. Effect of low level laser therapy on wound healing after palatal surgery in beagle dogs. *Lasers Surg Med* 1991: **11**: 462–470.

67. Jackson Z, Meghji S, MacRobert A, Henderson B, Wilson M. Killing of the yeast and hyphal forms of *Candida albicans* using a light-activated antimicrobial agent. *Lasers Med Sci* 1999: **14**: 150–157.

68. Jori G, Fabris C, Soncin M, Ferro S, Capellotti O, Dei D, Fantetti L, Chiti G, Roncucci G. Photodynamic therapy in the treatment of microbial infections: basic principles and perspective applications. *Lasers Surg Med* 2006: **38**: 468–481.

69. Jori G. Photodynamic therapy of microbial infections: state of the art and perspectives. *J Environ Pathol Toxicol Oncol* 2006: **25**: 505–520.

70. Kana JS, Hutschenreiter G, Haina D, Waidelich W. Effect of low power density laser radiation on healing of open skin wounds in rats. *Arch Surg* 1981: **116**: 293–296.

71. Karu T. Photobiology of low power laser effects. *Health Phys* 1989: **56**: 691–704.

72. Kimura YW, Wilder-Smith P, Yonaga K, Matsumoto K. Treatment of dentine hypersensivity by lasers: a review. *J Clin Periodontol* 2000: **27**: 715–721.

73. Kömerik N, Nakanishi H, MacRobert AJ, Henderson B, Speight P, Wilson M. In vivo killing of *Porphyromonas gingivalis* by toluidine blue-mediated photosensitization in an animal model. *Antimicrob Agents Chemother* 2003: **47**: 932–940.

74. Kömerik N, Wilson M. Factors influencing the susceptibility of Gram-negative bacteria to toluidine blue O mediated lethal photosensitization. *J Appl Microbiol* 2002: **92**: 618–623.

75. Kreisler M, Christoffers AB, Al-Haj H, Willershausen B, d'Hoedt B. Low-level 809 nm diode laser induced in vitro stimulation of the proliferation of human gingival fibroblasts. *Lasers Surg Med* 2002: **30**: 365–369.

76. Ladalardo TC, Pinheiro A, Campos RAC, Brugnera A Jr, Zanin F, Albernaz PLM, Weckx LL. Laser therapy in the treatment of dentine hypersensitivity. *Braz Dent J* 2004: **15**: 144–150.

77. Lizarelli RFZ, Miguel FAC, Freitas-Pontes KM, Villa GEP, Nunez SC, Bagnato VS. Dentin hypersensitivity clinical study comparing LILT and LEDT keeping the same irradiation parameters. *Laser Phys Lett* 2010: **7**: 805–811.

78. Lizarelli RFZ, Miguel FAC, Villa GEP, Carvalho-Filho E, Pelino JEP, Bagnato VS. Clinical effects of low-intensity laser vs light-emitting diode therapy on dentin hypersensivity. *J Oral Laser Appl* 2007: **7**: 129–136.

79. Loevschall H, Arenholt-Bindslev D. Effect of low-level diode laser irradiation of human oral mucosa fibroblasts in vitro. *Lasers Surg Med* 1994: **14**: 347–354.

80. Lowe AS, Walker MD, O'Byrne M, Baxter GD, Hirst DG. Effect of low intensity monochromatic light therapy (890 nm) on a radiation impaired, wound-healing model in murine skin. *Lasers Surg Med* 1998: **23**: 291–298.

81. MacRobert AM, Meghji S, Henderson B, Wilson M. Effect of dosimetric and physiological factors on the lethal photosensitization of *Porphyromonas gingivalis* in vitro. *Photochem Photobiol* 1997: **65**: 1026–1031.

82. Maegawa Y, Itoh T, Hosokawa T, Yaegashi K, Nishi M. Effects of near-infrared low-level laser irradiation on microcirculation. *Lasers Surg Med* 2000: **27**: 427–437.

83. Maiman TH. Stimulated optical radiation in ruby. *Nature* 1960: **187**: 493–494.

84. Maisch T. Anti-microbial photodynamictherapy: useful in the future? *Laser Med Sci* 2007: **22**: 83–91.

85. Malik Z, Ladan H, Nitzan Y. Photodynamic inactivation of Gram-negative bacteria: Problems and possible solutions. *J Photochem Photobiol, B* 1992: **14**: 262–266.

86. Marques MM, Pereira AN, Fujihara NA, Nogueira FN, Eduardo CP. Effect of low-power laser irradiation on protein synthesis and ultrastructure of human gingival fibroblasts. *Lasers Surg Med* 2004: **34**: 260–265.

87. Meisel P, Kocher T. Photodynamic therapy for periodontal diseases: state of the art. *J Photochem Photobiol, B* 2005: **79**: 159–170.

88. Melo MAS, Passos VF, Alves JJ, Barros EB, Santiago SL, Rodrigues LKA. Th effect of diode laser irradiation on dentin as a preventive measure against erosion: an *in vitro* study. *Lasers Med Sci* 2011: **26**: 615–621.

89. Mester E, Mester AF, Mester A. The biomedical effects of laser application. *Lasers Surg Med* 1985: **5**: 31–39.

90. Mester E. Effect of laser rays on wound healing. *Am J Surg* 1971: **122**: 532–535.

91. Mombelli A. Microbiology and antimicrobial therapy of peri-implantitis. *Periodontol 2000* 2002: **28**: 177–189.

92. Moore J, Wilson M, Kieser JB. The distribution of bacterial lipopolysaccharide (endotoxin) in relation to periodontally involved root surfaces. *J Clin Periodontol* 1986: **13**: 748–751.

93. Munin E, Giroldo LM, Alves LP, Costa MS. Study of germ tube formation by *Candida albicans* after photodynamic antimicrobial chemotherapy (PACT) . *J Photochem Photobiol B* 2007: **88**: 16–20.

94. Noro Filho GA, Casarin RC, Casati MZ, Giovani EM. PDT in non-surgical treatment of periodontitis in HIV patients: a split-mouth, randomized clinical trial. *Lasers Surg Med* 2012: **44**: 296–302.

95. Novaes AB Jr, Schwartz-Filho HO, de Oliveira RR, Feres M, Sato S, Figueiredo LC. Antimicrobial photodynamic therapy in the non-surgical treatment of aggressive periodontitis: microbiological profile. *Lasers Med Sci* 2012: **27**: 389–395.

96. Núñez SC, Nogueira GEC, Ribeiro MS, Garcez AS, Lage-Marques JL. He-Ne laser effects on blood microcirculation during wound healing: a method of in vivo sudy trough laser Doppler flowmetry. *Lasers Surg Med* 2004: **35**: 363–368.

97. Nyman S, Lindhe J, Karring T, Rylander H. New attachment following surgical treatment of human periodontal disease. *J Clin Periodontol* 1982: **9**: 290–296.

98. Oliveira RR, Novaes AB Jr, Garlet GP, Souza RF, Taba M Jr, Sato S, Souza S, Palioto DB, Grisi MF, Feres M. The effect of a single episode of antimicrobial photodynamic therapy in the treatment of experimental periodontitis. Microbiological profile and cytokine pattern in the dog mandible. *Lasers Med Sci* 2011: **26**: 359–367.

99. Oliveira RR, Schwartz-Filho HO, Novaes AB, Garlet GP, de Souza RF, Taba M, Scombatti de Souza SL, Ribeiro FJ. Antimicrobial photodynamic therapy in the non-surgical treatment of aggressive periodontitis: cytokine profile in gingival crevicular fluid, preliminary results. *J Periodontol* 2009: **80**: 98–105.

100. Oliveira RR, Schwartz-Filho HO, Novaes AB, Taba M. Antimicrobial photodynamic therapy in the non-surgical treatment of aggressive periodontitis: a preliminary ramdomized controlled clinical study. *J Periodontol* 2007: **78**: 965–973.

101. Pallasch TJ. Antibiotic resistance. *Dent Clin North Am* 2003: **47**: 623–639.

102. Peloi LS, Soares RR, Biondo CE, Souza VR, Hioka N, Kimura E. Photodynamic effect of light-emitting diode light on cell growth inhibition induced by methylene blue. *J Biosci* 2008: **33**: 231–237.

103. Pereira AN, Eduardo CP, Matson E, Marques MM. Effect of low-power laser irradiation on cell growth and procollagen synthesis of cultured fibroblasts. *Lasers Surg Med* 2002: **31**: 263–267.

104. Pereira CA, Romeiro RL, Cost ACBP, Machado AKS, Junqueira JC, Cardoso Jorge AO. Susceptibility of *Candida albicans, Staphylococcus aureus*, and *Streptococcus mutans* biofilms to photodynamic inactivation: an in vitro study. *Lasers Med Sci* 2011: **26**: 341–348.

105. Perez BA, Planet PJ, Kachlany SC, Tomich M, Fine DH, Figurski DH. Genetic analytic of the requirement for flp-2, tadV, and rcpB, in *Aggregatibacter actinomycetemcomitans* biofilm formation. *J Bacteriol* 2006: **188**: 6361–6375.

106. Pessoa ES, Melhado RM, Theodoro LH, Garcia VG. The histologic assessment of the influence of low-intensity laser therapy on wound healing in steroid-treated animals. *Photomed Laser Surg* 2004: **22**: 199–204.

107. Pfitzner A, Sigusch BW, Albrecht V, Glockmann E. Killing of periodontopathogenic bacteria by photodynamic therapy. *J Periodontol* 2004: **75**: 1343–1349.

108. Pinheiro ALB, Limeira Junior FA, Gerbi MEM, Ramalho LMP, Marzola C, Ponzi EAC. Effect of low level laser therapy on the repair of bone defects grafted with inorganic bovine bone. *Braz Dent J* 2003: **14**: 177–181.

109. Pinheiro ALB, Soares LGP, Aciole GTS, Correia NA, Barbosa AFS, Ramalho LMP, Santos JN. Light microscopic description of the effects of laser phototherapy on bone defects grafted with mineral trioxide aggregate, bone morphogenetic proteins, and guided bone regeneration in a rodent model. *J Biomed Mater Res* 2011: **98**: 212–221.

110. Pinheiro SL, Donegá JM, Seabra LM, Adabo MD, Lopes T, do CT, Ribeiro MC, Bertolini PF. Capacity of photodynamic therapy for microbial reduction in periodontal pockets. *Lasers Med Sci* 2010: **25**: 87–91.

111. Plaetzer K, Krammer B, Berlanda J, Berr F, Kiesslich T. Photophysics and photochemistry of photodynamic therapy: fundamental aspects. *Laser Med Sci* 2009: **24**: 259–268.

112. Prates RA, Silva EG, Yamada AM Jr, Suzuki LC, Paula CR, Ribeiro MS. Light parameters influence cell viability in antifungal photodynamic therapy in a fluence and rate fluence-dependent manner. *Laser Phys* 2009: **19**: 1038–1044.

113. Prates RA, Yamada AM, Suzuki LC, França CM, Cai S, Mayer MP, Ribeiro AC, Ribeiro MS. Histomorphometric and microbiological assessment of photodynamic therapy as an adjuvant treatment for periodontitis: a short-term evaluation of inflammatory periodontal conditions and bacterial reduction in a rat model. *Photomed Laser Surg* 2011: **29**: 835–844.

114. Prates RA, Yamada AM Jr, Suzuki LC, Hashimoto MCE, Cai S, Gouw-Soares S, Gomes L, Ribeiro MS. Bactericidal effect of malachite green and red laser on *Actinobacillus actinomycetemcomitans*. *J Photochem Photobiol B* 2007: **86**: 70–76.

115. Qin YL, Luan XL, Bi LJ, Sheng YQ, Zhou CN, Zhang ZG. Comparison of toluidine blue-mediated photodynamic therapy and conventional scaling treatment for periodontitis in rats. *J Periodontal Res* 2008: **43**: 162–167.

116. Raab O. The effect of fluorescent agents on infusoria. *Z Biol* 1900: **39**: 524–526.

117. Ramfjord SP, Caffesse RG, Morrison EC, Hill RW, Kerry GJ, Appleberry EA, Nissle RR, Stults DL. 4 modalities of periodontal treatment compared over 5 years. *J Clin Periodontol* 1987: **14**: 445–452.

118. Rams TE, Slots J. Local delivery of antimicrobial agents in the periodontal pocket. *Periodontol 2000* 1996: **10**: 139–159.

119. Renvert S, Wikström M, Dahlén G, Slots J, Egelberg J. Effect of root debridement on the elimination of *Actinobacillus actinomycetemcomitans* and *Bacteroides gingivalis* from periodontal pockets. *J Clin Periodontol* 1990: **17**: 345–350.

120. Romano AC, Aranha AC, Silveira BL, Baldochi SL, Eduardo CP. Evaluation of carbon dioxide laser irradiation associated with calcium hydroxide in the treatment of dentinal hypersensitivity. A preliminary study. *Lasers Med Sci* 2011: **26**: 35–42.

121. Rühling A, Fanghänel J, Houshmand M, Kuhr A, Meisel P, Schwahn C, Kocher T. Photodynamic therapy of persistent pockets in maintenance patients – a clinical study. *Clin Oral Invest* 2010: **14**: 637–644.

122. Rydén H, Persson L, Preber H, Bergström J. Effect of low power level energy laser irradiation on gingival inflammation. *Swed Dent J* 1994: **18**: 35–41.

123. Salmeron S, Rezende MLR, Consolaro A, Sant'Ana ACP, Damante CA, Greghi SLA, Passanezi E. Laser therapy as an effective method for implant surface decontamination: a histomorphometric study in rats. *J Periodontol* 2013: **84**: 641–649.

124. Sarkar S, Wilson M. Lethal photosensitization of bacteria in subgingival plaque from patients with chronic periodontitis. *J Periodontal Res* 1993: **28**: 204–210.

125. Sawasaki I, Geraldo-Martins VR, Ribeiro MS, Marques MM. Effect of low-intensity laser therapy on mast cell degranulation in juman oral mucosa. *Lasers Med Sci* 2009: **24**: 113–116.

126. Schwarz F, Arweiler N, Georg T, Reich E. Desensitizing effects of an Er:YAG laser on hypersensitive dentine – A

controlled, prospective clinical study. *J Clin Periodontol* 2002: **29**: 211–215.

127. Séguier S, Souza SL, Sverzut AC, Simioni AR, Primo FL, Bodineau A, Corrêa VM, Coulomb B, Tedesco AC. Impact of photodynamic therapy on inflammatory cells during human chronic periodontitis. *J Photochem Photobiol, B* 2010: **101**: 348–354.

128. Sgolastra FP, Petrucci A, Gatto R, Monaco A. Effectiveness of laser in dentinal hypersensitivity treatment: a systematic review. *J Endod* 2011: **37**: 297–303.

129. Shibli JA, Martins MC, Nociti FH Jr, Garcia VG, Marcantonio E Jr. Treatment of ligature-induced peri-implantitis by lethal photosensitization and guided bone regeneration: a preliminary histologic study in dogs. *J Periodontol* 2003: **74**: 338–345.

130. Shibli JA, Martins MC, Ribeiro FS, Garcia VG, Nociti FH Jr, Marcantonio E Jr. Lethal photosensitization and guided bone regeneration in treatment of periimplantitis: an experimental study in dogs. *Clin Oral Implants Res* 2006: **17**: 273–281.

131. Sigusch BW, Pfitzner A, Albrecht V, Glockmann E. Efficacy of photodynamic therapy on inflammatory signs and two selected periodontopathogenic species in a beagle dog model. *J Periodontol* 2005: **76**: 1100–1105.

132. Silva TC, Oliveira TM, Sakai VT, Dionísio TJ, Santos CF, Bagnato VS, Machado MAAM. In vivo effects on the expression of vascular endothelial growth factor-A165 messenger ribonucleic acid of an infrared diode laser associated or not with a visible red diode laser. *Photomed Laser Surg* 2010: **28**: 63–68.

133. Silveira LB, Prates RA, Novelli MD, Marigo HA, Garrocho AA, Amormn JC, Sousa GR, Pinotti M, Ribeiro MS. Investigation of mast cells in human gingiva following low-intensity laser irradiation. *Photomed Laser Surg* 2008: **26**: 315–321.

134. Skinner SM, Gage JP, Wilce PA, Shaw RM. A preliminary study of the effects of laser radiation on collagen metabolism in cell culture. *Aust Dent J* 1996: **41**: 188–192.

135. Smith K. The photobiological basis of low-level laser radiation therapy. *Laser Ther* 1991: **3**: 19–24.

136. Soares BM, Alves OA, Ferreira MV, Amorim JC, Sousa GR, de Silveira Lde B, Prates RA, Avila TV, Baltazar Lde M, de Souza Dda G, Santos DA, Modolo LV, Cisalpino PS, Pinotti M. *Cryptococcus gattii*: in vitro susceptibility to photodynamic inactivation. *Photochem Photobiol* 2011: **87**: 357–364.

137. Soares BM, daSilva DL, Sousa GR, Amorim JC, de Resende MA, Pinotti M, Cisalpino PS. In vitro photodynamic inactivation of Candida spp. growth and adhesion to buccal epithelial cells. *J Photochem Photobiol B* 2009: **94**: 65–70.

138. Soga Y, Saito T, Nishimura F, Ishimaru F, Mineshiba J, Mineshiba F, Takaya H, Sato H, Kudo C, Kokeguchi S, Fujii N, Tanimoto M, Takashiba S. Appearance of multidrug-resistant opportunistic bacteria on the gingiva during leukemia treatment. *J Periodontol* 2008: **79**: 181–186.

139. Soukos NS, Goodson JM. Photodynamic therapy in the control of oral biofilms. *Periodontol 2000* 2011: **55**: 143–166.

140. Soukos NS, Som S, Abernethy AD, Ruggiero K, Dunham J, Lee C, Doukas AG, Goodson JM. Phototargeting oral black pigmented bacteria. *Antimicrob Agents Chemother* 2005: **49**: 1391–1396.

141. Soukos NS, Wilson M, Burns T, Speight PM. Photodynamic effects of toluidine blue on human oral keratinocytes and fibroblasts and *Streptococcus sanguis* evaluated in vitro. *Lasers Surg Med* 1996: **18**: 253–259.

142. Souza SC, Junqueira JC, Balducci I, Koga-Ito CY, Munin E, Jorge AO. Photosensitization of different *Candida* species by low power laser light. *J Photochem Photobiol, B* 2006: **83**: 34–38.

143. Takamatsu N, Yano K, He T, Umeda M, Ishikawa I. Effect of initial periodontal therapy on the frequency of detecting *Bacteroides forsythus, Porphyromonas gingivalis* and *Actinobacillus actinomycetemcomitans. J Periodontol* 1999: **70**: 574–580.

144. Takasaki AA, Aoki A, Mizutani K, Schwarz F, Sculean A, Wang CY, Koshy G, Romanos G, Ishikawa I, Izumi Y. Application of antimicrobial photodynamic therapy in periodontal and peri-implant diseases. *Periodontol 2000* 2009: **51**: 109–1403.

145. Taylor R, Shklar G, Roeber F. The effects of laser radiation on teeth, dental pulp and oral mucosa of experimental animals. *Laser* 1965: **19**: 786–795.

146. Tenius FP, Biondo-Simões MLP, Ioshii SO. Effects of chronic use of dexamethasone on cutaneous wound healing in rats. *An Bras Dermatol* 2007: **82**: 141–149.

147. Theodoro LH, Sampaio JEC, Haypek P, Zezell BL, DM, Garvia VG. . Effect of Er:YAG and Diode lasers on the adhesion of blood components and on the morphology of irradiated root surfaces. *J Periodontal Res* 2006: **41**: 381–390.

148. Theodoro LH, Silva SP, Pires JR, Soares GH, Pontes AE, Zuza EP, Spolidório DM, de TB, Garcia VG. Clinical and microbiological effects of photodynamic therapy associated with nonsurgical periodontal treatment. A 6-month follow-up. *Lasers Med Sci* 2012: **27**: 687–693.

149. Theodoro LH, Zezell DM, Garcia VG, Haypek P, Nagata MJH, Almeida JM, Eduardo CP. Comparative analysis of root surface smear layer removal by different etching modalities or erbium: yttrium-aluminum-garnet laser irradiation. A scanning electron microscopy study. *Lasers Med Sci* 2010: **25**: 485–491.

150. Theodoro LH, Garcia VG, Haypek P, Zezell DM, Eduardo CP. Morphologic analysis, by means of scanning electron microscopy, of the effect of Er:YAG laser on root surfaces submitted to scaling and root planning. *Pesqui Odontol Bras* 2002: **16**: 308–312.

151. Torres CS, dos Santos JN, Monteiro JSC, Amorim PGM, Pinheiro ALB. Does the use of laser photobiomodulation, bone morphogenetic proteins, and guided bone regeneration improve the outcome of autologous bone grafts? *Photomed Laser Surg* 2008: **26**: 371–377.

152. Usacheva MN, Teichert MC, Biel MA. Comparison of the methylene blue and toluidine blue photobactericidal efficacy against gram-positive and gram-negative microorganisms. *Lasers Surg Med* 2010: **29**: 165–173.

153. Usacheva MN, Teichert MC, Biel MA. The interaction of lipopolysaccharides with phenothiazine dyes. *Lasers Surg Med* 2003: **33**: 311–319.

154. Vera DM, Haynes MH, Ball AR, Dai T, Astrakas C, Kelso MJ, Hamblin MR, Tegos GP. Strategies to potentiate antimicrobial photoinactivation by overcoming resistant phenotypes. *Photochem Photobiol* 2012: **88**: 499–511.

155. Wainwright M, Giddens RM. Phenothiazinium photosensitisers: choice in synthesis and application. *Dyes Pigm* 2003: **57**: 245–257.

156. Wainwright M, Phoenix DA, Gaskell M, Marshall B. Photobactericidal activity of methylene blue derivatives against vancomycin-resistant *Enterococcus* spp. *J Antimicrob Chemother* 1999: **44**: 823–825.

157. Wainwright M. Photodynamic antimicrobial chemotherapy (PACT). *J Antimicrob Chemother* 1998: **42**: 13–28.

158. Walker MD, Rumpf S, Baxter GD, Hirst DG, Lowe AS. Effect of low intensity laser irradiation (660 nm) on a radiation impaired wound-healing model in murine skin. *Lasers Surg Med* 2000: **26**: 41–47.

159. Walsh JT, Cummings JP. Effect of the dynamic optical properties of water on mid infrared laser ablation. *Lasers Surg Med* 1994: **15**: 295–305.

160. Weber JBB, Pinheiro ALB, Oliveira MG, Oliveira FAM, Ramalho LMP. Laser therapy improves healing of bone defects submitted to autologus bone graft. *Photomed Laser Surg* 2006: **24**: 38–44.

161. Wilson M, Dobson J, Sarkar S. Sensitization of periodontopathogenic bacteria to killing by light from a low-power laser. *Oral Microbiol Immunol* 1993: **8**: 182–187.

162. Wilson M. Lethal photosensitisation of oral bacteria and its potential application in the photodynamic therapy of oral infections. *Photochem Photobiol Sci* 2004: **3**: 412–418.

163. Wood S, Metcalf D, Devine D, Robinson C. Erythrosine is a potential photosensitizer for the photodynamic therapy of oral plaque biofilms. *J Antimicrob Chemother* 2006: **57**: 680–684.

164. Wood S, Nattress B, Kirkham J, Shore R, Brookes S, Griffiths J, Robinson C. An in vitro study of the use of photodynamic therapy for the treatment of natural oral plaque biofilms formed in vivo. *J Photochem Photobiol B Biol* 1999: **50**: 1–7.

165. Yin MT, Dobkin JF, Grbic JT. Epidemiology, pathogenesis, and management of human immunodeficiency virus infection in patients with periodontal disease. *Periodontol 2000* 2007: **44**: 55–81.

166. Yu W, Naim JO, Lanzafame RJ. Effects of photo stimulation on wound healing in diabetic mice. *Lasers Surg Med* 1997: **20**: 56–63.

167. Zanin ICJ, Brugnera A Jr, Zanin FAA, Teixeira A, Pratten J, Wilson M. Current stage of antimicrobial photodynamic therapy in Dentistry. In: Pinheiro ALBA, Zanin FAA, Brugnera A Jr, editors.*Application of Laser in Dentistry.*São Paulo: Santos Editora,2010: 153–167 (Portuguese).

168. Zapata HFN, Salmeron S, Silva AL, Damante CA, Sant'Ana ACP, Rezende MLR. Microbiological analysis of the effect of photodynamic therapy on decontamination of titanium surface. *Full Dent Sci* 2012: **3**: 279–284. (Portuguese).

169. Zeina B, Greenman J, Purcell WM, Das B. Killing of cutaneous microbial species by photodynamic therapy. *Br J Dermol* 2001: **144**: 274–278.

170. Zharikov EV, Zhecov VI, Kulevskii LA, Murina TM, Osiko VV, Prokhorov AM, Savel'ev AD, Smirnov VV, Starikov BP, Timoshechkin MI. Stimulated emission from Er^{3+} ions in yttrium aluminum garnet crystals at $\lambda = 2.94$ μ. *Sov. J Quantum Electron* 1975: **4**: 1039–1040.