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Application of antimicrobial photodynamic therapy in periodontal and peri-implant diseases

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Recent advances in technology have led to a constant drive to develop novel approaches for the treatment of periodontal diseases. The need to find more optimal treatment protocols for periodontal disease is a long-term goal for periodontal researchers and clinicians. A novel noninvasive photochemical approach for infection control, namely photodynamic therapy, has been receiving much attention in the treatment of oral diseases (34, 44, 142). Although the original technique was first employed in the treatment of cancer (4), during the last decade an increasing number of studies on photodynamic therapy application have been published in periodontics. They have reported efficient elimination of periodontal pathogens using the photodynamic method, which combines the application of a nontoxic chemical agent (photosensitizer) with low-level light energy (25, 37, 115). Photodynamic therapy has been considered as a promising novel therapeutic approach for eradicating pathogenic bacteria in periodontal and peri-implant diseases. In this review article, an overview on the existing preclinical and clinical evidence on the effects of photodynamic therapy in the treatment of periodontal and peri-implant diseases is presented and discussed.

Bacterial elimination using conventional methods in periodontal therapy

Periodontal disease results from inflammation of the supporting structures of the teeth in response to chronic infections caused by various periodontopathic bacteria (30). The main objective of periodontal therapy is to eliminate deposits of bacteria and bacterial niches by removing the supragingival and subgingival biofilm (126). Plaque removal with eradication of niches of causative pathogens is currently performed using mechanical methods, such as nonsurgical therapy, which results in significant clinical improvements and varying success rates (39). However, it has been demonstrated that conventional mechanical therapy cannot completely remove all periodontal pathogens; this is because of the anatomical complexity of the tooth roots, which may contain furcation areas and concavities, especially in deep periodontal pockets (2, 128), and the bacteria invading the surrounding soft tissues (5, 76, 127). Potential periodontal pathogens, such as *Aggregatibacter actinomycetemcomitans* and *Porphyromonas gingivalis* are capable of disrupting host epithelial cells and invading into deeper periodontal tissues (5, 76). Thus, recolonization by those bacteria remaining in pockets or the tissues after mechanical debridement is a problem but this should be prevented by periodontal therapy.

In order to facilitate reduction in the number of bacteria, antimicrobial or antiseptic agents are introduced into the periodontal pocket (97, 116). Bacterial infection may be well controlled when these agents are applied and thus supplemental chemotherapy is often recommended. Systemic use of antibiotics may be recommended in certain situations as an adjunct to periodontal therapy (97, 128). Local or systemic chemotherapy in conjunction with

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mechanical debridement (mechano-chemotherapy) are currently accepted approaches in the treatment of periodontal disease (68, 136). However, the use of antimicrobial agents suffers from two major drawbacks. The first is the difficulty experienced in maintaining stable therapeutic concentrations of the agent in the periodontal pocket for a sufficient length of time to ensure eradication of the organisms present, because the mixture of gram-positive and gram-negative bacteria grow as complex aggregates within a polymeric matrix (biofilms) on the surfaces of the teeth, leading to inhibition of the action of antimicrobial agents and antiseptics (119). The second drawback is the strong possibility of the development of resistance to antibiotics by the target organisms (135). Therefore, there has been significant interest in the development of new antimicrobial concepts, with fewer complications, as alternatives to conventional chemotherapy.

Since the beginning of the 1990s, the application of light energy (in other words, phototherapy) has been considered as a novel treatment approach in periodontics. In general, the use of lasers has been proposed as a new technical modality in the treatment of periodontal diseases (8, 52, 53). Dental lasers have been used as an effective means of decontamination of periodontal pockets over a period of 20 years. Lasers possess high bactericidal properties and they have demonstrated effective killing of oral pathogenic bacteria associated with periodontitis and peri-implantitis (7, 28, 82). Most high-level lasers exhibit bactericidal effects by thermal denaturation or direct ablation or destruction of bacterial cells and their application has been gradually increasing in daily clinical practice (10, 53). High-level laser systems are now applied as nonsurgical or surgical periodontal and peri-implant therapies (53, 110).

In spite of the substantial bactericidal effects of high-level lasers (7, 98), there is limited clinical evidence to demonstrate clearly that lasers can produce a greater reduction in the number of subgingival bacteria than that achieved using traditional mechanical therapy (29). Also, the use of high-level lasers usually results in irreversible thermal damage to the surrounding periodontal tissues and there is a concern of unexpected side effects, such as excessive ablation or thermal coagulation, carbonization or necrosis of the root, the gingival connective tissue, the bone and the pulp tissues, depending on the type of laser employed (8, 53, 137).

Recently, a new type of noninvasive phototherapy for bacterial elimination, called photodynamic therapy, has been introduced, which uses low-level laser light (69, 73, 134). Unlike high-level lasers, photodynamic therapy can selectively target the bacteria without potentially damaging the host tissues (49, 66, 83, 115). Photodynamic therapy has been extensively studied in the laboratory, and clinical trials have been recently initiated in the field of periodontics and peri-implant therapy.

Antimicrobial photodynamic therapy

Photodynamic therapy was discovered accidentally at the beginning of the 20th century (133) and was then applied in the medical field for the light-induced inactivation of cells, microorganisms or molecules (4, 69, 134). Photodynamic therapy basically involves three nontoxic ingredients: visible harmless light; a nontoxic photosensitizer; and oxygen. It is based on the principle that a photosensitizer (i.e. a photoactivatable substance) binds to the target cells and can be activated by light of a suitable wavelength. Following activation of the photosensitizer through the application of light of a certain wavelength, singlet oxygen and other very reactive agents are produced that are extremely toxic to certain cells and bacteria (69, 70, 111, 134).

Theoretically, neither the photosensitizer nor light alone can induce an efficient cytotoxic effect on the cells. The photosensitizer is generally applied in the targeted area by topical application, aerosol delivery or interstitial injection. The light that activates the photosensitizer must be of a specific wavelength with a relatively high intensity. With the discovery and development of lasers that are collimated, coherent and monochromatic, this therapy proved to be a great evolution because it became possible to utilize a homogeneous intensive light with low-level energy that was suitable for activation of the photodynamic reaction.

Photodynamic therapy has been applied in the medical field with different targets. One target is host mammalian tissue in the treatment of cancers (4). It has been shown that photosensitizers have a selective affinity for tumor or vascular tissue, and after excitation by light they produce cytotoxic effects, which may lead to cell death or tissue destruction by necrosis or apoptosis (24, 59). The other target recently broadly discussed is the microorganism. The microorganism is an important target in the treatment of local oral infections, and photodynamic therapy has been introduced as an important novel disinfection therapy in the field of dentistry. The

inactivation of microorganisms using photodynamic therapy has been defined as antimicrobial photodynamic therapy (69, 70), photodynamic antimicrobial chemotherapy (134) and photodynamic disinfection or lethal photosensitization.

Previous studies have demonstrated the simplicity of the technique and the efficient and beneficial bactericidal effect of antimicrobial photodynamic therapy (58, 115, 139, 140) in the treatment of periodontal infections. Antimicrobial photodynamic therapy can be easily applied, even in sites where there is limited access for mechanical instrumentation as a result of the anatomical complexity of the root and where remaining bacteria may be present. In addition, the antimicrobial effect of photodynamic therapy can be easily controlled by regulating the reaction; that is, by controlling the amount of light applied to activate the reaction. Using this simple procedure, bacteria can be eradicated in a very short period of time.

Mechanisms involved in antimicrobial photodynamic therapy

The proposed mechanisms of photodynamic antimicrobial reactions at the molecular level have already been explained in previous important reviews (38, 59, 69, 134). The bactericidal effect of photodynamic therapy can be explained by two potential, but different, mechanisms. One is DNA damage (41) and the other is the damage caused to the cytoplasmic membrane of the bacteria by cytotoxic species generated by antimicrobial photodynamic therapy (13), leading to events such as inactivation of the membrane transport system, inhibition of plasma membrane enzyme activities, lipid peroxidation and others (12, 55, 79). Although it has been reported that antimicrobial photodynamic therapy can lead to DNA damage, it seems that bacterial killing by the photochemical reaction is mainly caused by damage to the bacterial cytoplasmic membrane (13, 48, 103).

The mechanism of action of antimicrobial photodynamic therapy can be briefly described as follows: after irradiation with light of a specific wavelength (lasers), the photosensitizer at ground state is activated to a highly energized triplet state (Fig. 1). The longer lifetime of the triplet state enables the interaction of the excited photosensitizer with the surrounding molecules, and it is generally accepted that the generation of cytotoxic species produced during photodynamic therapy occurs in this state (86). The triplet-state photosensitizer follows two different pathways (type I and II) to react with biomolecules (43, 111, 134).

Type I reactions involve hydrogen-atom abstraction or electron-transfer reactions between the excited state of the photosensitizer and an organic substrate molecule of the cells, which produces free radicals and radical ions. These free-radical species are generally highly reactive and interact with endogenous molecular oxygen to produce highly reactive oxygen species such as superoxide, hydroxyl radicals and hydrogen peroxide, which are harmful to cell membrane integrity, causing irreparable biological damage (43, 111).

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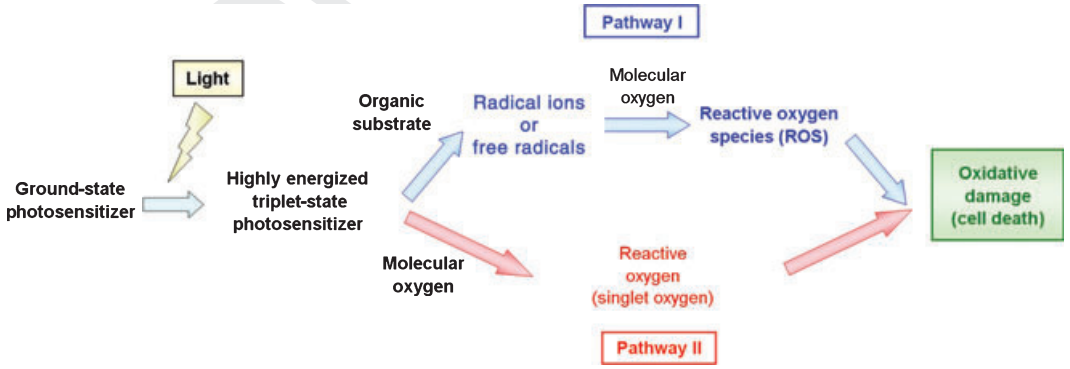

Fig. 1. Mechanism of photodynamic antimicrobial reactions at the molecular level. After irradiation with light of a specific wavelength, the photosensitizer in the ground state is converted to a highly-energized triplet state. The triplet-state photosensitizer follows two different pathways (I and II) to react with biomolecules. Pathway I involves the production of ions or electron / hydrogen removal from an organic substrate molecule of the cells to form free radicals. Pathway II involves the production of a highly reactive state of oxygen, known as singlet oxygen ($^1O_2$), which reacts with the surroundings as a result of its high chemical reactivity. The free radicals and the singlet oxygen convey toxic or lethal effects to the bacterial cell by damaging the cell membrane and the cell wall (111, 134).

In the type II reaction, the triplet-state photosensitizer reacts with oxygen to produce an electronically excited and highly reactive state of oxygen, known as singlet oxygen ($^1O_2$), which can interact with a large number of biological substrates as a result of its high chemical reactivity, inducing oxidative damage and ultimately lethal effects upon the bacterial cell by damaging the cell membrane and cell wall (43, 111). Microorganisms that are killed by singlet oxygen include viruses, bacteria, protozoa and fungi. Singlet oxygen has a short lifetime in biological systems (<0.04 ms) and a very short radius of action (0.02 μm) (78). Because of the limited migration of singlet oxygen from its site of formation as a result of its short lifetime, sites of initial cell damage from photodynamic therapy are closely related to the localization of the photosensitizer. Thus, the reaction takes place within a limited space, leading to a localized response and making it ideal for application at localized sites without affecting distant molecules, cells or organs (78, 93).

It seems that the primary cytotoxic agent responsible for the biological effects of the photo-oxidative process is singlet oxygen. Thus, the process of antimicrobial photodynamic therapy is generally mediated by a type II reaction, which is accepted as the major pathway in microbial cell damage (111, 134).

Photodynamic therapy in the treatment of oral diseases

Application of photodynamic therapy has led to significant advances in dentistry because the delivery of light is more accessible and topical application of the photosensitizer is more feasible in the oral cavity. Photodynamic therapy is used in the treatment of different types of oral solid tumors, and investigations into the application of photodynamic therapy to treat superficial precancerous oral lesions, such as oral leukoplakia, oral erythroleukoplakia and oral verrucous hyperplasia, have been widely performed, with some success (40, 63, 144). In addition, photodynamic therapy has been effectively applied in the treatment of lichen planus (3, 132).

Furthermore, the antimicrobial properties of photodynamic therapy make it a potential candidate for the treatment of bacterial, fungal and viral infections of the oral cavity. In operative dentistry, it has been well proven that the antimicrobial photodynamic therapy technique is effective for the treatment and prevention of dental caries. Several *in vitro* studies have demonstrated a strong bactericidal action of antimicrobial photodynamic therapy against gram-positive bacteria such as *Streptococcus sorbrinus*, *Streptococcus mutans* and *Streptococcus sanguinis*, which play an important role in the etiology of dental caries (91, 138, 145). Clinical trials of antimicrobial photodynamic therapy have been performed to eliminate the bacteria in softened carious dentine, thus intervening in the step-wise excavation techniques that may reduce the risk of pulpal exposure and necrosis, as well as the need for pulp capping (19).

In endodontics, antimicrobial photodynamic therapy has been reported to be effective as an adjunct to conventional endodontic disinfection treatment to destroy the bacteria that remain even after irrigation with sodium hypochlorite (18). Several studies demonstrated that antimicrobial photodynamic therapy was effective in eliminating anaerobic and aerobic bacteria, including *Enterococcus faecalis*, and *Actinomyces*, *Porphyromonas* and *Prevotella* spp., in primary endodontic lesions or in cases of endodontic treatment failure (42, 44, 45).

In addition, several studies have demonstrated that antimicrobial photodynamic therapy is highly effective in the destruction of *Candida albicans*, which is responsible for oropharyngeal candidiasis (36, 125, 140). Antimicrobial photodynamic therapy has also been reported to be successful in treating viral infections, including common labial herpes simplex infection, as it has been demonstrated ultrastructurally that the viral envelope which protected the virus from adsorption or penetration is photodamaged following antimicrobial photodynamic therapy (117, 118).

Antimicrobial photosensitizing agents and the wavelengths used in periodontal and peri-implant therapy

For the elimination of supragingival and subgingival plaque, antimicrobial photodynamic therapy has been applied with various combinations of lasers and photosensitizing agents. In antimicrobial photodynamic therapy, the particular photosensitizers employed are toluidine blue O [tolonium chloride: (7-amino-8-methyl-phenothiazin-3-ylidene)-dimethyl-ammonium ($C_{15}H_{16}N_3S+$)], methylene blue [3,7-bis(dimethyl-amino)phenazathionium chloride tetramethylthionine chloride ($C_{16}H_{18}N_3ClS$) or phenothiazine-5-ium, 3,7-bis(dimethylamino)-chloride],

erythrosine, chlorine e6 and hematoporphyrin, which have been shown to be safe when employed in the medical field.

The phenothiazine dyes (toluidine blue O and methylene blue) are the major photosensitizers applied clinically in the medical field. Both have similar chemical and physicochemical characteristics. Toluidine blue O is a solution that is blue–violet in color. It can stain granules within mast cells and proteoglycans and glycosaminoglycans within connective tissues. In the field of oral surgery, toluidine blue O has been used to detect mucosal tumors or atypical epithelia as normal mucosal epithelium cannot be stained by toluidine blue O (67). Methylene blue is a redox indicator that is blue in an oxidizing environment and becomes colorless upon reduction. In medical practice, methylene blue is applied for identification of dysplasias or precancerous lesions of the mucosa (87). Recently, because of the photocatalytic action of methylene blue, it has been utilized for virus inactivation in blood plasma before blood transfusions, using a white fluorescent lamp (64). Methylene blue combined with light has also been reported to be beneficial in killing the influenza virus (64), *Helicobacter pylori* (77) and *C. albicans* (140).

With respect to antimicrobial photodynamic therapy, it has been demonstrated that methylene blue and toluidine blue O are very effective photosensitizing agents for the inactivation of both gram-positive and gram-negative periodontopathic bacteria (25, 58, 102, 139). There is, however, a difference in susceptibility of gram-positive and gram-negative bacteria to treatment. Anionic and neutral photosensitizers are reported to be effective against gram-positive bacteria; however, they are often ineffective against gram-negative bacteria (71, 74). Although it is still a point of debate, gram-negative organisms seem to be generally more resistant to photodynamic therapy than gram-positive bacteria, as a result of the differences in the outer membrane structures of both types of bacteria (69, 101). Gram-positive species have a relatively porous cytoplasmic membrane that permits entry of the photosensitizer into the cell (71). In gram-negative species, an additional outer membrane layer with a characteristic structure works as an effective permeability barrier that inhibits the penetration of host cellular and humoral defense factors and may lead to resistance against many antibiotics (85). Thus, the outer membrane may reduce or prevent photosensitizer uptake. However, it has been demonstrated that photosensitizers, such as toluidine blue O and methylene blue, which undergo a pronounced cationic charge, can bind to the outer membrane of gram-negative bacteria and penetrate bacterial cells (15, 75, 131), demonstrating a high degree of selectivity for killing microorganisms compared with host mammalian cells (120). Therefore, toluidine blue O and methylene blue have been the photosensitizers of choice in the treatment of periodontitis and peri-implantitis. However, toluidine blue O seems to exhibit a greater ability for killing gram-positive and gram-negative bacteria than methylene blue. Elimination of *A. actinomycetemcomitans*, *P. gingivalis* and *Fusobacterium nucleatun* has been demonstrated to be more effectively achieved whilst using toluidine blue O than methylene blue (139). It has been shown *in vitro* that toluidine blue O interacts with lipopolysaccharide more effectively than does methylene blue (130), thus a greater photobactericidal effect of toluidine blue O against gram-negative bacteria can be expected than for methylene blue (129).

In the past, photosensitizer activation was achieved by a variety of light sources such as argon lasers (50), potassium titanyl phosphate (90) or neodymium-doped: yttrium, aluminum and garnet (Nd:YAG) lasers (23). Currently, however, the light sources of a specific wavelength mostly applied in photodynamic therapy are those of helium–neon lasers (633 nm), gallium-aluminum-arsenide diode lasers (630–690, 830 or 906 nm) and argon lasers (488–514 nm), the wavelengths of which range from visible light to the blue of argon lasers, or from the red of helium–neon and gallium–aluminum–arsenide lasers to the infrared area of some diode lasers. High-level-energy laser irradiation is not used to activate the photoactive dye because relatively low-level exposure produces a high bactericidal effect. Several types of laser devices have been applied during *in vitro* research studies. However, in the case of *in vivo* and clinical investigations, the diode lasers are the light source predominantly applied (Table 1). Although toluidine blue O was generally selected as the photosensitizer of choice in previous *in vitro* studies, methylene blue has been used mainly in clinical studies because clinical photodynamic therapy kits that include methylene blue are already commercially available (Periowave™; Ondine Biopharma Corporation, Vancouver, Canada) (Helbo®; Photodynamic Systems GmbH & Co. KG, Grieskirchen, Austria). Recently, nonlaser light sources, such as light-emitting diodes, have been suggested as new light activators in photodynamic therapy as light-emitting diode devices are more compact and portable and the cost is much lower compared with that of traditional lasers.

Table 1. Antimicrobial photosensitizing agents and the wavelengths used in periordontal and peri-implant therapy

Photosensitizer	Type of study	Concentration of the photosensitizer	Light (wavelength)	Light parameters and (time of exposure)	Author and year (reference)
Toluidine blue O	*In vitro*	0.005%	He-Ne laser (632.8 nm)	7.3 mW (10 and 30 s)	Dobson & Wilson 1992 (35)
	In vitro	25 μg/ml	He-Ne laser (632.8 nm)	7.3 mW (80 s)	Wilson et al. 1993 (139)
	In vitro	50 μg/ml	He-Ne (632.8 nm)	7.3 mW, 30 s	Sarkar & Wilson 1993 (102)
	In vitro	100 μg/ml	He/Ne laser (632.8 nm) and Diode laser (660 nm)	7.3 mW and 11 mW, respectively	Wilson et al. 1995 (141)
	In vitro	12.5, 25, 50 μg/ml	He-Ne (632.8 nm)	7.3 mW	Bhatti et al. 1997 (14)
	In vitro	100 μg/ml	Diode laser (905 nm)	7.3 mW, 60 s	Haas et al. 1997 (46)
	In vitro	100 μg/ml	He-Ne (632.8 nm)	7.3 mW	Bhatti et al. 2002 (17)
	In vitro	25 μg/ml	He-Ne laser (632 nm)	35 mW (15 min)	O'Neill et al. 2002 (88)
	In vitro	50 μg/ml	He-Ne laser (635 nm) and Red filtered Xenon lamp	10, 25 and 100 mW/cm^2	Matevski et al. 2003 (72)
	In vitro	10, 100, 500, 1000 and 2500 μg/ml	Diode laser (635 nm)	260 mW: 53, 106, 159 and 212 mW/cm^2 (14–226 s)	Qin et al. 2008 (95)
	In vivo (mice)	1 mg/ml	Diode laser (635 nm)	CW, 61 mW (377 s)	Luan et al. 2007 (66)
	In vivo (rat)	25, 50 and 200 μg/ml	Diode laser (633 nm)	100 mW (5, 8 and 16 min)	Kömerik et al. 2002 (57)
	In vivo (rat)	0.01, 0.1 and 1 mg/ml	Diode laser (630 nm)	100 mW (1, 2, 4 and 8 min)	Kömerik et al. 2003 (58)
	In vivo (rat)	1 mg/ml	Diode laser (635 nm)	CW, 61 mW (75 s)	Qin et al. 2008 (96)
	In vivo (rat)	100 μg/ml	Diode laser (685 nm)	50 mW (120 s)	de Almeida et al. 2008 (32)
	In vivo (dog, peri-implantitis)	100 μg/ml	Diode laser (685 nm)	50 mW (80 s)	Shibli et al. 2003 (112)
	In vivo (dog, peri-implantitis)	100 μg/ml	Diode laser (685 nm)	50 mW (80 s)	Shibli et al. 2003 (113)
	In vivo (dog, peri-implantitis)	100 μg/ml	Diode laser (830 nm)	CW, 50 mW (80 s)	Shibli et al. 2006 (114)
	Clinical (peri-implantitis)	100 μg/ml	Diode laser (906nm)	60 s	Haas et al. 2000 (47)
	Clinical (peri-implantitis)	100 μg/ml	Diode laser (690 nm)	60 s	Dörtbudak et al. 2001 (37)

Table 1. *Continued*

Photosensitizer	Type of study	Concentration of the photosensitizer	Light (wavelength)	Light parameters and (time of exposure)	Author and year (reference)
Methylene blue	*In vitro*	0.005%	He-Ne laser (632.8 nm)	7.3 mW (10 and 30 s)	Dobson & Wilson 1992 (35)
	In vitro	25 μg/ml	He-Ne laser (632.8 nm)	7.3 mW (80 s)	Wilson et al. 1993 (139)
	In vitro	0.01% (w/v)	He-Ne laser (632.8 nm), Diode laser (665 and 830 nm)	30 mW (30 s) and 100 mW (100 s), respectively	Chan & Lai 2003 (25)
	In vivo (rat)	100 μg/ml	Diode laser (685 nm)	50 mW (120 s)	de Almeida et al. 2007 (31)
	In vivo (rat)	100 μg/ml	Diode laser (685 nm)	50 mW (120 s)	de Almeida et al. 2008 (32)
	Clinical (periodontitis)	0.005% (w/v)	Diode laser (685 nm)	CW, 30 mW (71 s)	Yilmaz et al. 2002 (143)
	Clinical (periodontitis)	0.005% (w/v)	Diode laser (670nm)	CW, 150 mW	Andersen et al., 2007 (6)
	Clinical (periodontitis)	10 mg/ml	Diode laser (660 nm)	CW, 60 mW (60 s)	de Oliveira et al. 2007 (34)
	Clinical (periodontitis)	10 mg/ml	Diode laser (660 nm)	CW, 100 mW (60 s)	Braun et al., 2008 (20)
	Clinical (periodontitis)	10 mg/ml	Diode laser (670 nm)	75 mW (60 s)	Chondros et al. 2008 (26)
	Clinical (periodontitis)	10 mg/ml	Diode laser (670 nm)	75 mW (60 s)	Christodoulides et al. 2008 (27)
Poly-L-lysine (pL) - chlorin *e*6 (ce6) conjugate	*In vitro*	5 μM	Diode laser (671 nm)	230 mW (10 min)	Soukos et al. 1998 (121)
	In vitro	5 μM	Diode laser (662 nm)	25 mW	Soukos et al. 2003 (122)
Chlorin e6	*In vivo* (dogs)	N.a.	Diode laser (662nm)	500 mW (20 s)	Sigusch et al. 2005 (115)
BLC1010	*In vivo* (dogs)	N.a.	Diode laser (662nm)	500 mW (20 s)	Sigusch et al. 2005 (115)
Phthalocyanine	*In vitro*	0.005%	He-Ne laser (632.8 nm)	7.3 mW (10 and 30 s)	Dobson & Wilson 1992 (35)
Aluminium disulphonated phthalocyanine (AlPcS2)	*In vitro*	100 μg/ml	He/Ne laser (632.8 nm) and Diode laser (660 nm)	7.3 mW and 11 mW, respectively	Wilson et al. 1995 (141)
Hematoporphyrin ester	*In vitro*	0.005%	He-Ne laser (632.8 nm)	7.3 mW (10 and 30 s)	Dobson & Wilson 1992 (35)
Hematoporphyrin HCl	*In vitro*	0.005%	He-Ne laser (632.8 nm)	7.3 mW (10 and 30 s)	Dobson & Wilson 1992 (35)

Table 1. *Continued*

Photosensitizer	Type of study	Concentration of the photosensitizer	Light (wavelength)	Light parameters and (time of exposure)	Author and year (reference)
Hematoporphyrin oligomers	*In vivo* (mouse)	2.5, 10, and 20 mg / kg body weight	Diode laser (630 nm)	1. ED = 90 or 180 J / cm^2, pulsed 2. ED = 5 mJ / cm^2, pulsed 3. ED = 15 mJ / cm^2, CW	Pe et al. 1993 (92)
Azulene	*In vivo* (dogs)	25% (w / v)	Diode laser (660 nm)	40 mW (180 s)	Hayek et al. 2005 (49)
Endogenous porphyrins	*In vitro*	–	Argon laser (488–514 nm)	0.58 mW	Henry et al. 1995 (50)
	In vitro	–	Argon laser (488–514 nm)	0.58 mW	Henry et al. 1996 (51)
	In vitro	–	Broadband light (380–520)	70 mW / cm^2	Soukos et al. 2005 (123)

CW, continuous-wave; ED, energy density; He-Ne, helium–neon; NA, not available; w / v, weight / volume.

Antimicrobial photodynamic therapy in the treatment of periodontal and peri-implant diseases

Based on the advantages and characteristics of antimicrobial photodynamic therapy, it has been proposed that periodontal and peri-implant diseases are potential targets of this novel antimicrobial photochemotherapy. Antimicrobial photodynamic therapy is expected to resolve the difficulties and problems of conventional antimicrobial therapy and can work as an adjunctive to conventional mechanical treatments.

The photosensitizer is placed directly in the periodontal and peri-implant pocket and the liquid agent can easily access the whole root or implant surface before activation by the laser light through placement of the optical fiber directly in the pocket (Fig. 2). As a result of the technical simplicity of the method and the high effectiveness of bacterial killing, the application of antimicrobial photodynamic therapy in the treatment of periodontal and peri-implant diseases has recently been studied extensively.

In vitro studies of the antimicrobial effects of photodynamic therapy in periodontal therapy

The bactericidal effect of antimicrobial photodynamic therapy on periodontal pathogens has been demonstrated in several basic studies (Table 2). In the early 1990s, Dobson and Wilson (35) showed that low-level helium–neon laser irradiation with toluidine blue O or methylene blue was effective for killing *P. gingivalis*, *F. nucleatum*, *A. actinomycetemcomitans* and *S. sanguinis*. Compared with other photosensitizers, toluidine blue O and methylene blue were more effective for killing periodontal pathogens in antimicrobial photodynamic therapy (139). These authors also revealed that the most effective bactericidal effect was achieved with the combination of toluidine blue O and a helium–neon laser in a supragingival biofilm model study (141). Bhatti et al. (14) demonstrated that the optimal concentration of toluidine blue O to kill *P. gingivalis* was 12.5 μg / ml with helium–neon laser irradiation. In addition, they revealed, by transmission electron microscopic examination, that the bactericidal effect of light-activated toluidine blue O against *P. gingivalis* was caused by disruption of the outer membrane proteins of those bacteria (17). Chan and Lai

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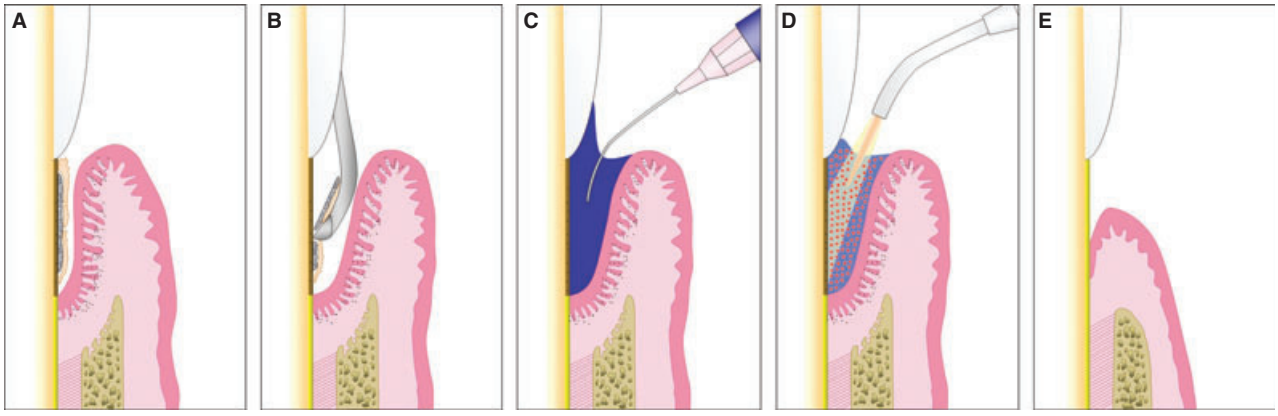

Fig. 2. Diagram showing the steps of application of antimicrobial photodynamic therapy in the treatment of periodontitis. (A) Periodontally diseased site before treatment. (B) Mechanical debridement using hand curettes. (C) Application of the photosensitizer via syringe at the diseased site that contains residual bacteria. Occasionally, excess dye solution is removed using water spray. (D) Photosensitization is performed using an intensive light by a special tip applied in the pocket. Singlet oxygen and other very reactive agents that are toxic to bacteria are produced, resulting in photochemical disinfection of the periodontal pocket. (E) Improved wound healing in the treated site.

(25) showed that in the presence of methylene blue, the wavelengths of 632.8 nm (helium–neon laser) and 665 and 830 nm (diode laser) had a high bactericidal effect on periodontal pathogens. Matevski et al. (72) reported that even a conventional light (red-filtered xenon lamp) could be as effective as lasers in the antimicrobial effect of photodynamic therapy using toluidine blue O. Soukos et al. (121) demonstrated that using a cationic poly-L-lysine–chlorin e6 conjugate, photodynamic therapy could kill *P. gingivalis* and *Actinomyces viscosus* without causing epithelial cell damage, whilst photodynamic therapy with anionic conjugates could not achieve elimination of bacteria.

Moreover, the bactericidal effect of antimicrobial photodynamic therapy was demonstrated not only on pure cultures of bacteria but also on the plaque biofilm. Sarkar and Wilson (102) reported that helium–neon laser irradiation combined with toluidine blue O killed oral bacteria within samples of subgingival plaque obtained from patients with chronic periodontitis. O'Neil et al. (88) also demonstrated that the combination of helium–neon laser irradiation and toluidine blue O was effective at decreasing
20 the number of different species of bacteria in biofilms prepared from the saliva of healthy subjects. Recently, Qin et al. (95) investigated the optimal parameters required for effective antimicrobial photodynamic therapy-induced killing of supragingival periodontal pathogens using the combination of different toluidine blue O concentrations and laser-irradiation energies and reported that diode laser irradiation at 12 J/cm^2 with 1 mg/ml of toluidine blue O was the most effective option. In addition, Soukos et al. (122) demonstrated the bactericidal effects of photodynamic therapy with poly-L-lysine–chlorin e6 conjugate and a diode laser against subgingival plaque biofilm that comprised both gram-positive and gram-negative bacteria. They demonstrated that the bacteria present in the deep layers of the biofilm were killed by extensive penetration of the photosensitizer into the biofilm
21 following antimicrobial photodynamic therapy.

In black-pigmented bacteria such as *P. gingivalis* and *Prevotella* spp., the endogenous porphyrins present on the bacteria may also act as a photosensitizer. Henry et al. (50) reported that, without a dye agent, argon laser irradiation could kill black-pigmented bacteria and that *P. gingivalis* was the species of bacteria most sensitive to photodynamic therapy. They also reported that argon laser irradiation without dye agents effectively killed black-pigmented bacteria formed within the biofilm (51). Recently, Soukos et al. (123) also demonstrated that irradiation with nonlaser light (broadband light: 380–520 nm) had a bactericidal effect against black-pigmented bacteria and that the effect depended upon the quality of endogenous porphyrins.

In addition, it seems that antimicrobial photodynamic therapy not only kills the bacteria but may also lead to the detoxification of endotoxins because it has been demonstrated *in vitro* that lipopolysaccharide treated by photodynamic therapy did not stimulate the production of pro-inflammatory cytokines by mononuclear cells (56); thus, photodynamic therapy may inactivate endotoxins such as lipopolysaccharide by decreasing their biological activity.

Table 2. *In vitro* studies on the bactericidal and inactivation effects of photodynamic therapy in periodontics.

Author and year (reference)	Sample	Light (wavelength)	Photosensitizer (concentration)	Light parameters and time of exposure	Purpose of application	Findings
Dobson & Wilson 1992 (35)	Cultured biofilm	He-Ne laser (632.8 nm)	TBO, MB, phthalocyanine, hematoporphyrin HCl and hematoporphyrin ester (0.005, 0.005, 0.005, 0.005 and 0.005% respectively)	7.3 mW 10 and 30 s	Bactericidal effect on microorganism in cultured biofilm (*P.g.*, *F.n.*, *A.a.* and *S.s.*)	TBO and MB were more effective at killing bacteria compared with other photosensitizers during PDT
Wilson et al. 1993 (139)	Cell suspension	He-Ne laser (632.8 nm)	TBO and MB (25 μg/ml and 25 μg/ml, respectively)	7.3 mW 80 s	Bactericidal effect on microorganism in cell suspension (*P.g.*, *F.n.* and *A.a.*)	Low doses of laser light are effective at killing bacteria
Sarkar & Wilson 1993 (102)	Subgingival plaque	He-Ne laser (632.8 nm)	TBO (100 mg/ml)	7.3 mW 30 s	Bactericidal effect on subgingival plaque sample containing *P.g.*, *F.n.* and *Streptococci*	The dye/laser combination achieved significant reductions in the viability of aerobes, anaerobes and, especially, BPB anaerobes
Wilson et al. 1995 (141)	Supragingival plaque	He-Ne laser (632.8 nm) and diode laser (660 nm)	TBO and aluminium disulfonated phthalocyanine (100 μg/ml and 100 μg/ml, respectively)	7.3 mW and 11 mW, respectively	Bactericidal effect on supragingival plaque sample containing *Streptococci* and *Actinomyces*	The He-Ne/TBO combination was more effective than the GaAs/AlPcS2 combination
Henry et al. 1995 (50)	Cell suspension	Argon laser (488 and 514 nm)	Endogenous porphyrins	0.58 mW	Bactericidal effect on BPB (*P.g.* and *Prevotella* ssp.)	*P.g.* was the most sensitive to argon laser irradiation. For elimination of BPB, oxygen was required during laser irradiation. Non-BPB were much less sensitive to irradiation than BPB
Henry et al. 1996 (51)	Cultured biofilm	Argon laser (488 and 514 nm)	Endogenous porphyrins	0.58 mW	Bactericidal effect on BPB (*P.g.* and *Prevotella* ssp.)	The biofilms of *P.g.*, *P.n.* and *P.m.* were susceptible to argon laser without the addition of an exogenous photosensitizer

Table 2. *Continued*

Author and year (reference)	Sample	Light (wavelength)	Photosensitizer (concentration)	Light parameters and time of exposure	Purpose of application	Findings
Bhatti et al. 1997 (14)	Cell suspension	He-Ne laser (632.8 nm)	TBO (12.5, 25, 50 μg/ml)	7.3 mW	Determine the effect of dosimetric and physiological factors on the lethal photosensitization of *P.g.*	Light dose-dependent increase in killing bacteria. No significant effect on the numbers killed when the concentration of TBO was increased from 12.5 to 50 mg/ml
Soukos et al. 1998 (121)	Cell suspension	Diode laser (671 nm)	Poly-L-lysine–chlorin e6 conjugate (5 μM)	230 mW 10 min	Bactericidal effect on microorganism in suspension (*P.g.* and *A.v.*) and cultured oral epithelial cells	PDT with the cationic conjugate killed 99% of bacteria, while oral epithelial cells remained intact
Bhatti et al. 2002 (17)	Cell suspension	He-Ne laser (632.8 nm)	TBO (100 μg/ml)	7.3 mW	Assessment of the PDT effect on cytoplasmic membrane	Light-activated TBO caused bacterial death, affecting the permeability of the bacterial membrane
O'Neill et al. 2002 (88)	Biofilm prepared from saliva	He-Ne laser (632 nm)	TBO (25 μg/ml)	35 mW 15 min	Bactericidal effect on oral bacterial biofilm	Substantial numbers of oral bacteria in multispecies biofilms could be killed by light in the presence of TBO
Chan & Lai 2003 (25)	Cell suspension	He-Ne laser (632.8 nm) and diode laser (665 and 830 nm)	MB 0.01% (w/v)	30 mW (30 s) and 100 mW (100 s), respectively	Bactericidal effect on cultured microorganism (*P.i.*, *F.n.*, *A.a.* and *S.s.*)	Exposure of bacterial cultures to PDT using MB results in a dose-dependent decrease in viability. The most effective combination was that of MB and 655-diode laser at 100 mW
Matevski et al. 2003 (72)	Cell suspension	He-Ne laser, red-filtered xenon lamp (635 nm)	TBO (50 μg/ml)	Light intensity: 10, 25 and 100 mW/cm^2	Comparison of xenon lamp and He-Ne laser in combination with TBO to suppress *P. g.*	PDT utilizing a conventional light source is effective in suppressing *P.g. in vitro* in the presence of serum and blood

Table 2. *Continued*

Author and year (reference)	Sample	Light (wavelength)	Photosensitizer (concentration)	Light parameters and time of exposure	Purpose of application	Findings
Soukos et al. 2003 (122)	Cell suspension prepared from subgingival plaque	Diode laser (662 nm)	Poly-L-lysine–chlorin e6 conjugate (5 μM)	Light intensity: 25 mW/cm^2	Bactericidal effect on subgingival plaque sample and measurement of the depth of photosensitizer penetration into biofilm	PDT enabled almost 90% killing of bacteria on dental plaque. Penetration depth of the photosensitizer into the biofilm increased following laser irradiation
Soukos et al. 2005 (123)	Subgingival plaque and cell suspension	Broadband light (380–520 nm)	Endogenous porphyrins	Light intensity: 70 mW/cm^2	Bactericidal effect on cultured BPB (*P.g.*, *P.i.*, *P.n.*, *P.m.* and *S.c.*)	Higher cell death was observed on the bacteria with higher concentation of endogenous porphyrins
Qin et al. 2008 (95)	Cell suspension prepared from supragingival plaque	Diode laser (635 nm)	TBO (10, 100, 500, 1000 and 2500 μg/ml)	260 mW; light intensity: 53, 106, 159 and 212 mW/cm^2 14–226 s	Comparison of different parameters on bactericidal effect of supragingival plaque sample from periodontal patients	The best bactericidal effect was observed in the treatment with 1 mg/ml TBO at 159 mW/cm^2 light irradiation. The effect was different among the plaque samples employed

AlPcS2, aluminium disulphonated phthalocyanine; BPB, black-pigmented bacteria; CW, continuous wave; GaAs, xxxxxx xxxxxxxxx; He-Ne, helium–neon; MB, methylene blue; PDT, photodynamic therapy; TBO, toluidine blue O; w/v, weight/volume.
A.a., *Aggregatibacter actinomycetemcomitans*; *A.v.*, *Actinomyces viscosis*; *F.n.*, *Fusobacterium nucleatum*; *P.g.*, *Porphyromonas gingivalis*; *P.i.*, *Prevotella intermedia*; *P.m.*, *Prevotella melaninogenica*; *P.n.*, *Prevotella nigrescens*; *S.c.*, *Streptococcus constellatus*; S.s., *Streptococcus sanguis*; T.d., *Treponema denticola*.

As discussed previously, analysis of a number of *in vitro* studies supports the contention that antimicrobial photodynamic therapy with specific photosensitizers and light sources is effectively bactericidal for periodontal pathogens. However, the most effective combination of wavelengths and photosensitizers, as well as the optimal parameters required (such as agent concentration and agent exposure time, laser power energy and irradiation time), have not yet been elucidated and therefore more basic studies are still necessary to optimize clinical application.

In vitro studies of the antimicrobial effects of photodynamic therapy in periodontal therapy

Recently, animal studies have been performed to help clarify the clinical response to antimicrobial photodynamic therapy application in periodontal therapy (Table 3). Some animal studies have reported
22 a reduction in the microbial load in ligature-induced periodontitis following the application of photodynamic therapy. Kömerik et al. (58) demonstrated that a significant reduction in the *P. gingivalis* count was detected after the treatment of experimentally induced periodontitis in rats using toluidine blue O in combination with a diode laser. Sigusch et al. (115) showed that the chlorin-e6 plus diode laser also achieved a reduction in the *P. gingivalis* count in dogs, but failed to reduce the number of
23 *F. nucleatum*.
24 Following a reduction in the microbial load in periodontal diseases, improvements in signs of clinical inflammation, such as redness and bleeding on probing, were also demonstrated. Both toluidine blue O-mediated photodynamic therapy used in rats (96) and chlorin-e6-mediated photodynamic therapy
25 applied in dogs (115), exhibited positive results. Qin et al. (96) reported a significant reduction in the total bacterial flora and, histologically, a large reduction in inflammatory cell infiltration after application of antimicrobial photodynamic therapy (toluidine blue O + diode laser) in the treatment of experimentally induced periodontitis in rats. Comparing the photosensitization of periodontal bacteria with scaling and root planing, the clinical and histological improvements, as well as bacterial elimination, following photodynamic therapy gave results similar to those of conventional scaling. Sigusch et al. (115) demonstrated that antimicrobial photodynamic therapy (chlorin-e6 and BLC1010 + diode
26 laser) was distinctly advantageous in reducing the periodontal signs of redness and bleeding on prob-
27 ing in dogs and resulted in significant suppression of *P. gingivalis*.

Regarding the effect of antimicrobial photodynamic therapy on bone levels, Kömerik et al. (58) demonstrated, in a histological examination using rats, that, 90 days post-treatment, toluidine blue O-mediated photodynamic therapy had induced a decrease in alveolar bone loss around teeth with experimentally induced periodontitis. de Almeida et al. (31) compared, histologically and radiographically, the progression of experimentally induced periodontitis after treatment with methylene blue alone, low-level laser therapy alone, or with methylene blue followed by low-level laser therapy (pho-
28 todynamic therapy). The results of radiographic evaluation demonstrated that photodynamic therapy had a short-term effect (up to 15 days) upon the reduction of periodontal tissue destruction. However, at 30 days there were no significant differences between the groups. de Almeida et al. (32) also compared the effect of toluidine blue O, low-level laser therapy and photodynamic therapy treatments on the bone loss of periodontally affected furcations in rats. The photodynamic therapy showed a short-term effect (up to 15 days) upon decreasing bone loss, but no significant differences between groups were observed at 30 days post-therapy. In addition, de Almeida et al. (33) confirmed that adjunctive antimicrobial photodynamic therapy led to significant reductions in periodontal bone loss in diabetic rats, suggesting that antimicrobial photodynamic therapy might also be an effective adjunctive to conventional mechanical treatment in diabetic patients.

Generally, antimicrobial photodynamic therapy appears to suppress periodontal pathogens and to reduce signs of inflammation effectively and safely in periodontitis *in vivo*. However, there is a lack of evidence to prove that antimicrobial photodynamic therapy is capable of suppressing periodontopathogens in a single dose or course. Further *in vivo* studies investigating the antimicrobial effects on different periodontal pathogens need to be performed. The use of antimicrobial photodynamic therapy may reduce signs of periodontal inflammation and alveolar bone loss in experimentally induced periodontitis. However, two studies have shown a tendency for regression within 30 days after treatment in the effects on bone levels. Consequently, the long-term therapeutic outcomes should be further evaluated in animal models. The limited number of *in vivo* studies available indicates that antimicrobial

Table 3. *In vivo* studies on the bactericidal effects and safety of photodynamic therapy in periodontics.

Author and year (reference)	Animal (n)	Light (wavelength)	Photosensitizer (concentration)	Light parameters and time of exposure	Purpose of application	Findings
Pe et al. 1993 (92)	Mouse (5–7)	Nd:YAG laser (630 nm)	Hematoporphyrin oligomers (2.5, 10, and 20 mg/kg body weight)	Pulsed and CW, 5, 7.5 and 15 mJ/cm^2 20 and 60 min	Investigate the effects of PDT on the normal mouse tongue	PDT was provided safely to the mouse tongue
Kömerik et al. 2002 (57)	Rat (3)	Diode laser (633 nm)	TBO (25, 50 and 200 μg/ml)	100 mW 5, 8 and 16 min	Investigate the effect of TBO-mediated photosensitization on the rat buccal mucosa	No necrotic or inflammatory changes were found in the buccal mucosa following any of the treatments (using up to 200 μg/ml of TBO and 16 min of irradiation)
Kömerik et al. 2003 (58)	Rat (6)	Diode laser (630 nm)	TBO (0.01, 0.1 and 1 mg/ml)	100 mW 1, 2, 4 and 8 min	Investigate the microbial reduction (*P.g.*) in experimentally induced periodontitis	TBO-mediated lethal photosensitization of *P. g.* is possible *in vivo*, resulting in decreased levels of bone loss. No adverse effect of PDT on the adjacent tissues were observed
Sigusch et al. 2005 (115)	Dog (2)	Diode laser (662 nm)	Chlorin e6 and BLC1010	CW, 500 mW 20 s per tooth	Investigate the microbial reduction (*P.g.* and *F.n.*) in experimentally induced periodontitis	Reduction in the clinical inflammatory signs of redness and BOP, significant reduction in *P. gingivalis*, but *F. nucleatum* was hardly reduced with chlorine e6
de Almeida et al. 2007 (31)	Rat (10)	Diode laser (685 nm)	MB (100 μg/ml)	50 mW 60 s per site	Investigate the progression of experimentally induced periodontitis after PDT	Up to 15 days postoperatively the PDT group showed less bone loss compared with control, LLLT and MB groups. No difference between groups was observed at 30 days post-therapy. PDT had a short-term effect on the reduction of periodontal tissue destruction

Table 3. *Continued*

Author and year (reference)	Animal (n)	Light (wavelength)	Photosensitizer (concentration)	Light parameters and time of exposure	Purpose of application	Findings
Luan et al. 2007 (66)	Mouse (10)	Diode laser (635 nm)	TBO (1 g/ml)	CW, 61 mW 377 s per site	Investigate whether TBO-mediated photosensitization exerted damaging effects on periodontal tissues	No necrotic or inflammatory changes were found in the gingiva, dentin, dental pulp or alveolar bone
Qin et al. 2008 (96)	Rat (8)	Diode laser (635 nm)	TBO (1 mg/ml)	CW, 61 mW 75 s per site	Compare the microbial reduction and clinical improvements following PDT or SRP in the treatment of periodontitis	The signs of inflammation that accompanied periodontitis, such as redness, increased PI and GI values, BOP and inflammatory cell infiltration were greatly reduced. No detectable injury to host tissues was observed following therapy
de Almeida et al. 2008 (32)	Rat (10)	Diode laser (685 nm)	MB (100 μg/ml)	50 mW 60 s per site	Investigate histometrically the effect of PDT on bone loss in furcation areas	Up to 15 days postoperatively, the PDT group showed less bone loss in furcation areas compared with control, LLLT and MB groups. No difference was observed between the groups at 30 days post-therapy
de Almeida et al. 2008 (33)	Rat (10)	Diode laser (660 nm)	TBO (100 μg/ml)	30 mW 399 s per tooth	Investigate histomorphometrically the effect of PDT as an adjunctive to SRP in treatment of experimentally induced periodontitis in diabetic rats	In nondiabetes and diabetes groups, the animals treated by PDT showed significantly lower bone loss in all experimental periods than SRP, TBO and LLLT. The periodontal ligament appeared intact and inflammatory infiltrate was absent. The bone tissue showed no signs of resorption

BOP, bleeding on probing; CW, continuous wave; GBR, guided bone regeneration; GI, gingival index; LLLT, low-level laser therapy; MB, methylene blue; Nd:YAG, neodymium-doped: yttrium, aluminium and garnet; PDT, photodynamic therapy; PI, plaque index; SRP, scaling and root planing; TBO, toluidine blue.
A.a., Aggregatibacter actinomycetemcomitans; *F.n., Fusobacterium nucleatum*; *P.g., Porphyromonas gingivalis*; *P.i., Prevotella intermedia*; *P.n., Prevotella nigrescens.*

photodynamic therapy may be an alternative treatment to scaling.

Clinical studies of application of antimicrobial photodynamic therapy in the treatment of periodontal disease

Currently, five studies are available reporting on the use of antimicrobial photodynamic therapy as an adjunct to nonsurgical treatment for initial (6, 20, 27, 143) and maintenance (26) therapy of chronic periodontitis. In addition, one study has reported on the use of nonsurgical therapy in aggressive periodontal disease (34) (Tables 4 and 5) (Fig. 3).

Yilmaz et al. (143) randomly assigned a total of ten patients to receive repeated application of scaling and root planing + photodynamic therapy (methylene blue + 30 mW diode laser), scaling and root planing alone, photodynamic therapy alone or supragingival oral hygiene instructions. Methylene blue served as the photosensitizer and was used as a mouth rinse. Scaling and root planing was performed on days 1 and 7, while the laser was repeatedly applied over each papillary region (not into periodontal pockets) on days 1, 2, 4, 7, 9 and 11. After 32 days of healing, significant clinical and microbiological improvements were only observed in the scaling and root planing + photodynamic therapy and scaling and root planing alone groups. By contrast, improvements following photodynamic therapy treatment alone, as well in those receiving oral hygiene instructions, did not reach statistical significance. Regarding laser treatment, there were no complaints (such as discomfort, sensitivity or pain) from subjects immediately after therapy or at 3 weeks post-therapy. The authors concluded that antimicrobial photodynamic therapy provided no additional microbiological and clinical benefits over conventional mechanical debridement. The reduced effectiveness of photodynamic therapy in this study may be a result of the indirect application of photodynamic therapy from the external surface of the gingiva.

Two very recent randomized controlled clinical studies have evaluated the short-term clinical effects (up to a period of 3 months) of adjunctive antimicrobial photodynamic therapy to scaling and root planing in patients with chronic periodontitis (6, 20). Andersen et al. (6), using a parallel three-arm design, compared the effectiveness of antimicrobial photodynamic therapy with that of scaling and root planing for nonsurgical treatment of moderate to advanced periodontal disease. A total of 33 patients were assigned to photodynamic therapy alone (methylene blue + 50 mW diode laser), scaling and root planing alone or scaling and root planing + photodynamic therapy. Clinical assessments of bleeding on probing, probing pocket depth and clinical attachment level were made. After three months of healing it was observed that a combination of scaling and root planing + photodynamic therapy resulted in significant improvements in the investigated parameters over the use of scaling and root planing alone at all evaluation time points.

Braun et al. (20) evaluated the effect of adjunctive antimicrobial photodynamic therapy (methylene blue + 100 mW diode laser) in chronic periodontitis using a split-mouth design. A total of twenty patients received a scaling and root planing procedure and the quadrants were randomly assigned to an additional treatment with photodynamic therapy. Following irrigation after a residence time of 3 mins, the remaining photosensitizer was activated for 10 s per site (six sites in total). After 3 months of healing, the adjunctive use of photodynamic therapy resulted in a significantly higher change in mean relative attachment level, probing pocket depth, sulcus fluid flow rate and bleeding on probing at the sites receiving photodynamic therapy than at the sites receiving scaling and root planing alone. Accordingly, it was concluded that the clinical outcomes of conventional scaling and root planing may be improved by adjunctive antimicrobial photodynamic therapy in patients with chronic periodontitis.

Christodoulides et al. (27) evaluated the clinical and microbiological effects of the adjunctive use of antimicrobial photodynamic therapy (methylene blue + 75 mW diode laser) to nonsurgical periodontal treatment. A total of twenty-four patients suffering from chronic periodontitis were randomly assigned to either scaling and root planing followed by a single application of photodynamic therapy, or scaling and root planing alone. The photosensitizer was applied to the instrumented sites and thoroughly rinsed with sterile saline after 3 mins. The fiber tip was moved circumferentially around the tooth for 1 min, as recommended by the manufacturer. After 3 and 6 months of healing, both treatment procedures resulted in statistically and clinically significant reductions in mean probing pocket depth and clinical attachment level. However, no statistically significant differences in terms of clinical attachment level and probing pocket depth changes were found between the two groups. Similarly, both treatment procedures revealed comparable microbiological changes in common

Table 4. Clinical studies on the application of photodynamic therapy in the treatment of periodontal disease.

Author and year (reference)	Type of study (number of subjects)	Light (wavelength)	Photosensitizer Concentration, time of application	Light parameters and time of exposure method of irradiation	Purpose of application (period of observation)	Findings
Yilmaz et al. 2002 (143)	RCT, SMD (10)	Diode lase (685 nm)	MB 0.005% (w/v), 1 min	Pulsed, 30 mW (5 Hz) 71 s per each papillary region over gingiva	Initial therapy for chronic periodontitis (32 days)	Significant clinical and microbiological improvements were only observed in the SRP + PDT and SRP groups
Andersen et al. 2007 (6)	RCT (33)	Diode laser (670 nm)	Phenazathionium chloride* (MB) 0.005% (w/v)	CW, 150 mW 60 s per site into periodontal pockets	Initial therapy for chronic periodontitis (3 months)	SRP + PDT resulted in significant clinical improvements over SRP
de Oliveira et al. 2007 (34)	RCT (10)	Diode laser (660 nm)	Phenotizaine chloride† (MB) 10 mg/ml, 1 min	CW, 60 mW 60 s per tooth into periodontal pockets	Initial therapy for aggressive periodontitis (3 months)	Comparable clinical outcomes for PDT monotherapy and SRP
Braun et al. 2008 (20)	RCT, SMD (20)	Diode laser (660 nm)	Phenotizaine chloride† (MB) 10 mg/ml, 3 min	CW, 100 mW 60 s per tooth into periodontal pockets	Initial therapy for chronic periodontitis (3 months)	SRP + PDT resulted in significantly higher change in mean RAL than SRP
Chondros et al. 2008 (26)	RCT (24)	Diode laser (670 nm)	Phenotizaine chloride† (MB) 10 mg/ml, 3 min	CW, 75 mW 60 s per tooth into periodontal pockets	Maintenance therapy for chronic periodontitis (6 months)	SRP + PDT resulted in PD reduction and CAL gain comparable to SRP, but significantly higher reduction in mean bleeding scores than SRP
Christodoulides et al. 2008 (27)	RCT (24)	Diode laser (670 nm)	Phenotizaine chloride† (MB) 10 mg/ml, 3 min	75 mW 60 s per tooth into periodontal pockets	Initial therapy for chronic periodontitis (6 months)	SRP + PDT resulted in PD reduction and CAL gain comparable to SRP, but significantly higher reduction in mean bleeding scores than SRP

CAL, clinical attachment level; OHI, oral hygiene instruction; PPD, probing pocket depth; RAL, relative attachment level; RCT, randomized clinical trial; SMD, split-mouth design; SRP, scaling and root planing; w/v, weight/volume.
* Periowave™ Treatment Kit: 0.005% (w/v) [3,7-bis(dimethyl-amino)phenazathionium chloride trihydrate] Ondine Biopharma Corporation, Vancouver, BC, Canada; †Helbo® Blue Photosensitizer: 10 mg/ml of phenotizaine chloride [phenothiazine-5-ium, 3,7-bis(dimethylamino)-chloride] HELBO® Photodynamic Systems GmbH & Co KG, Grieskirchen, Austria.

Table 5. Clinical results of the studies (shown in Table 4) on the application of photodynamic therapy in the treatment of periodontal disease.

Author and year (reference)	Observation period			BOP reduction		
Yilmaz et al. 2002 (143)	32 days	SRP		50 ± 25 %		NS
		SRP + PDT		60 ± 28 %		
Andersen et al. 2007 (6)	3 months	SRP		56% (mean)		NS
		SRP + PDT		59% (mean)		
de Oliveira et al. 2007 (34)	3 months			**BOP**		
			Baseline		**3 months**	
		SRP	60%	NS	21%	NS
		PDT	57%		19%	
Braun et al. 2008 (20)	3 months	SRP		24% (median) (IQR: 21, min 2-max 61)		P < 0.05
		SRP + PDT		19% (median) (IQR: 11, min 2-max 64)		
Chondros et al. 2008 (26)	6 months			**BOP**		
			Baseline		**6 months**	
		SRP	58 ± 34 %		48 ± 36 %	P = 0.027
		SRP + PDT	69 ± 26 %		19 ± 22 %	
Christodoulides et al. 2008 (27)	6 months			**FMBS**		
			Baseline		**6 months**	
		SRP	59 ± 21 %	NS	20 ± 4 %	P < 0.05
		SRP + PDT	54 ± 16 %		10 ± 5 %	

Table 5. *Continued*

Author and year (reference)	Observation period			PPD reduction		
Yilmaz et al. 2002 (143)	32 days	SRP		0.49 ± 0.29 mm		NS
		SRP + PDT		0.66 ± 0.43 mm		
Andersen et al. 2007 (6)	3 months	SRP		0.74 ± 0.43 mm		P < 0.05
		SRP + PDT		1.11 ± 0.53 mm		
de Oliveira et al. 2007 (34)	3 months			**PPD**		
			Baseline		**3 months**	
		SRP	4.92 ± 1.14 mm	NS	3.98 ± 1.76 mm	NS
		PDT	4.92 ± 1.61 mm		3.49 ± 0.98 mm	
Braun et al. 2008 (20)	3 months	SRP		3.7 mm (median) (IQR: 0.6, min 3.4-max 6.0)		P < 0.05
		SRP + PDT		3.6 mm (median) (IQR: 0.6, min 3.2-max 5.3)		
Chondros et al. 2008 (26)	6 months	SRP	0.90 ± 0.80 mm			NS
		SRP + PDT	0.80 ± 0.50 mm			
Christodoulides et al. 2008 (27)	6 months	SRP	0.70 ± 0.70 mm			NS
		SRP + PDT	0.90 ± 0.30 mm			

Table 5. *Continued*

Author and year (reference)	Observation period			AL gain		
Yilmaz et al. 2002 (143)	32 days			N.a.		
Andersen et al. 2007 (6)	3 months	SRP	0.36 ± 0.35 mm			P < 0.02
		SRP + PDT	0.86 ± 0.61 mm			
de Oliveira et al. 2007 (34)	3 months			**AL**		
			Baseline		**3 months**	
		SRP	10.53 ± 2.30 mm	NS	9.01 ± 3.05 mm	NS
		PDT	9.93 ± 2.10 mm		8.74 ± 2.12 mm	
Braun et al. 2008 (20)	3 months	SRP		0.35 mm (median) (IQR: 0.21, min 0.11-max 0.81)		P < 0.05
		SRP + PDT		0.67 mm (median) (IQR: 0.36, min 0.20-max 1.89)		
Chondros et al. 2008 (26)	6 months	SRP	0.50 ± 0.60 mm			NS
		SRP + PDT	0.70 ± 0.70 mm			
Christodoulides et al. 2008 (27)	6 months	SRP	0.50 ± 0.50 mm			NS
		SRP + PDT	0.70 ± 0.30 mm			

AL, attachment level; BOP, bleeding on probing; FMBS, full-mouth bleeding score; IQR, interquatile range; max, maximum; min, minimum; NA, not available; NS, not significant; PDT, photodynamic therapy; PPD, probing pocket depth; SRP, scaling and root planing.

COLOUR

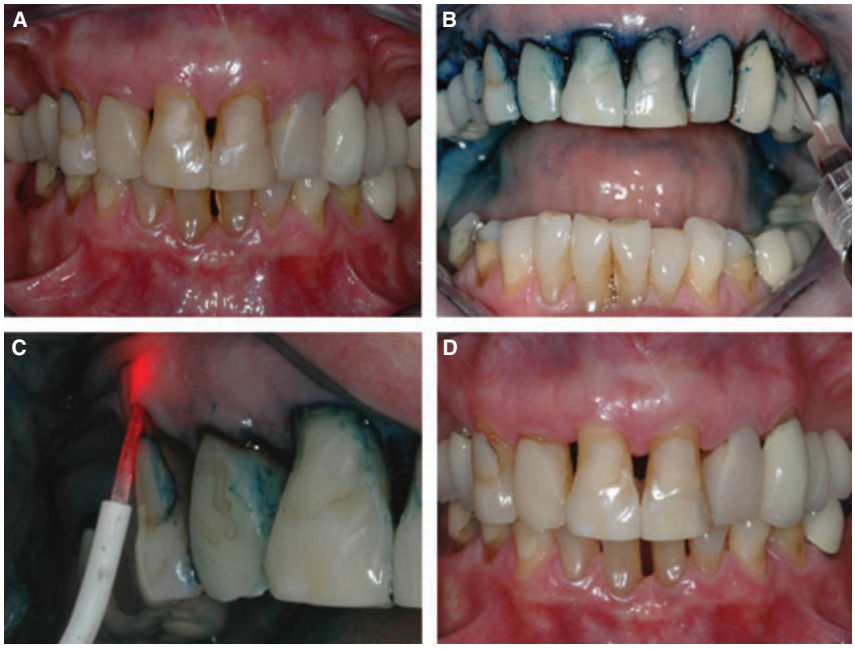

Fig. 3. Clinical application of antimicrobial photodynamic therapy in the treatment of periodontitis. (A) Clinical situation of a 51-year-old woman before nonsurgical periodontal therapy and antimicrobial photodynamic therapy. Full-mouth bleeding scores were 67%. The clinical parameters of the mesio-buccal site of the upper right lateral incisor were a probing pocket depth of 7 mm, clinical attachment level of 9 mm and gingival recession of 2 mm. The disto-palatal site of the upper left canine had a probing pocket depth of 9 mm and clinical attachment level of 9 mm without gingival recession. (B) Application of the photosensitizer following supragingival and subgingival mechanical debridement using curettes and the ultrasonic scaler. The photosensitizer applied was a 'Phenothiazine Chloride' (HELBO® Blue Photosensitizer, HELBO® Photodynamic Systems GmbH & Co. KG, Grieskirchen, Austria). The photosensitizer was kept in the periodontal pockets for 3 mins. (C) Irradiation with the diode laser. Laser irradiation was performed using a diode laser of 670 nm wavelength at 75 mW of power output (HELBO® TheraLite Laser, HELBO® Photodynamic Systems GmbH & Co. KG, Grieskirchen, Austria). Laser irradiation was performed for 1 min. (D) The clinical situation 6 months after therapy. The full-mouth bleeding scores were reduced to 15%. The mesio-buccal site of the upper right lateral incisor showed a pocket reduction of 3 mm, with 3 mm of attachment gain without gingival recession. The disto-palatal site of the upper left canine presented 4 mm of pocket reduction and 4 mm of attachment gain without causing any gingival recession. Significant clinical improvements of periodontal pockets were obtained with antimicrobial photodynamic therapy adjunctive to mechanical root debridement. (Operator: A.S.)

periodontal pathogens. However, at 3 and 6 months, the test group exhibited a significantly higher improvement in mean full-mouth bleeding scores, which might be partly attributed to the additional photo-biomodulation effect mediated by the low-level laser irradiation during photodynamic therapy (94). Based on these findings, it was concluded that a single episode of photodynamic therapy, as an adjunct to scaling and root planing, failed to result in an additional improvement in terms of probing pocket depth reduction and clinical attachment level gain. However, it resulted in a significantly higher reduction in bleeding scores, which should be taken into consideration under clinical conditions (27). Similar results were also observed when the same device was used as an adjunct to nonsurgical periodontal treatment in patients on periodontal maintenance in a study reported by Chondros et al. (26).

Only one study, by de Oliveira et al. (34), reported on the outcome of antimicrobial photodynamic therapy monotherapy for the treatment of aggressive periodontitis. A total of 10 patients were randomly assigned, according to a split-mouth design, to either photodynamic therapy (methylene blue + 60 mW diode laser) or scaling and root planing. Laser application was performed for 10 s per site after 3 mins of residence time of the photosensitizer. Three months later, both treatment procedures gave comparable clinical outcomes, as evidenced by probing pocket depth reductions and clinical attachment level gains, suggesting a potential clinical effect of photodynamic therapy as an alternative to scaling and root planing. In both groups, the beneficial effects were more pronounced at initially moderate and shallow pockets.

Taken together, the data available from controlled clinical studies indicate that in patients with chronic

periodontitis, the adjunctive use of antimicrobial photodynamic therapy to scaling and root planing may result, on a short-term basis (up to 3 or 6 months), in (i) higher reductions in bleeding on probing compared with scaling and root planing (as observed in four studies) and (ii) higher probing pocket depth reductions and clinical attachment level gains compared with scaling and root planing alone (in two studies). When interpreting the available data, it should be kept in mind that the evidence from randomized controlled clinical studies, evaluating the potential clinical benefit of photodynamic therapy in the treatment of periodontitis, is still limited. The main drawbacks may be related to the rather limited number of patients, the short-term duration of studies (i.e. 3 or 6 months) and the nonestablishment of the most effective procedure of antimicrobial photodynamic therapy. The available data seem to indicate that the adjunctive use of antimicrobial photodynamic therapy in nonsurgical periodontal therapy may improve the clinical outcome, but further studies are warranted before definitive conclusions can be drawn on the clinical relevance of antimicrobial photodynamic therapy in periodontal therapy.

Furthermore, recently, Brink and Romanos compared the clinical and microbiological effects of scaling and root planing + Nd:YAG laser (2W), scaling and root planing + 980 nm diode laser (2W), and scaling and root planing + antimicrobial photodynamic therapy [methylene blue + 670 nm diode laser (75 mW)] and scaling and root planing alone in patients with chronic periodontitis (21, 22) (published in German). The authors reported that in the group treated with antimicrobial photodynamic therapy + scaling and root planing, bleeding on probing was reduced significantly more, one to three months following treatment, than in the other groups. In addition, the bactericidal effects of scaling and root planing + antimicrobial photodynamic therapy appeared to be greater than those of the scaling and root planing + Nd:YAG laser, scaling and root planing + diode laser, or scaling and root planing alone treatments.

Application of antimicrobial photodynamic therapy in the treatment of peri-implant disease

Treatment of peri-implantitis has become an interesting topic among clinicians and researchers. With the extensive increase in placement of dental implants, the number of implants affected by peri-implantitis has also been increasing in clinical practice. In the treatment of peri-implantitis, it has been proven that complete eradication of the causative bacteria, which are similar to the pathogens responsible for the development of periodontal disease (65, 80, 81), and disinfection and detoxification of the diseased implant surface, as well as of the peri-implant pockets, are essential to achieve effective healing with regeneration of the lost bone around the affected implants. Conventional mechanical methods are apparently ineffective for complete debridement of the bone defect as well as of the contaminated microstructured implant surface (11, 54, 105). Thus, adjunctive application of systemic or local antibiotics and antiseptics has been generally recommended (100, 106, 109). However, because of the potential problems related to antibiotics (such as resistance) and antiseptics, as mentioned previously (135), and the generally insufficient bacterial irradiation as well as poor re-osseointegration following its adjunctive application during nonsurgical and surgical therapy of peri-implantitis, novel approaches are still necessary in the treatment of peri-implant diseases.

Recently, several studies have demonstrated bactericidal and detoxification effects of high-level lasers on contaminated dental implant surfaces (62, 109, 124). High-level lasers have been used successfully in the surgical management of peri-implantitis (109, 124). However, in nonsurgical therapy, high-level lasers have shown limited clinical efficacy (107, 108). Moreover, following the application of some lasers, surface alterations (such as melting and carbonization) have been observed on the treated titanium surface (61, 84, 89, 99). Antimicrobial photodynamic therapy was recently proposed as an adjunctive for bacterial elimination in the treatment of peri-implantitis, based on its successful application in the treatment of periodontitis (Fig. 4). Currently, one *in vitro*, four animal and two clinical studies are available reporting the various effects of application of antimicrobial photodynamic therapy as an adjunctive to the treatment of peri-implantitis (Table 6).

In an *in vitro* study, Hass et al. (46) examined the efficacy of antimicrobial photodynamic therapy in killing bacteria associated with peri-implantitis, such as *A. actinomycetemcomitans*, *P. gingivalis* or *Prevotella intermedia* (*P. intermedia*), which adhered to titanium plates with different surface characteristics. The plates were incubated with those bacteria and then subjected to four different treatments: (i) photodynamic therapy (toluidine blue O + diode laser); (ii) no treatment; (iii) laser light alone; and (iv) toluidine

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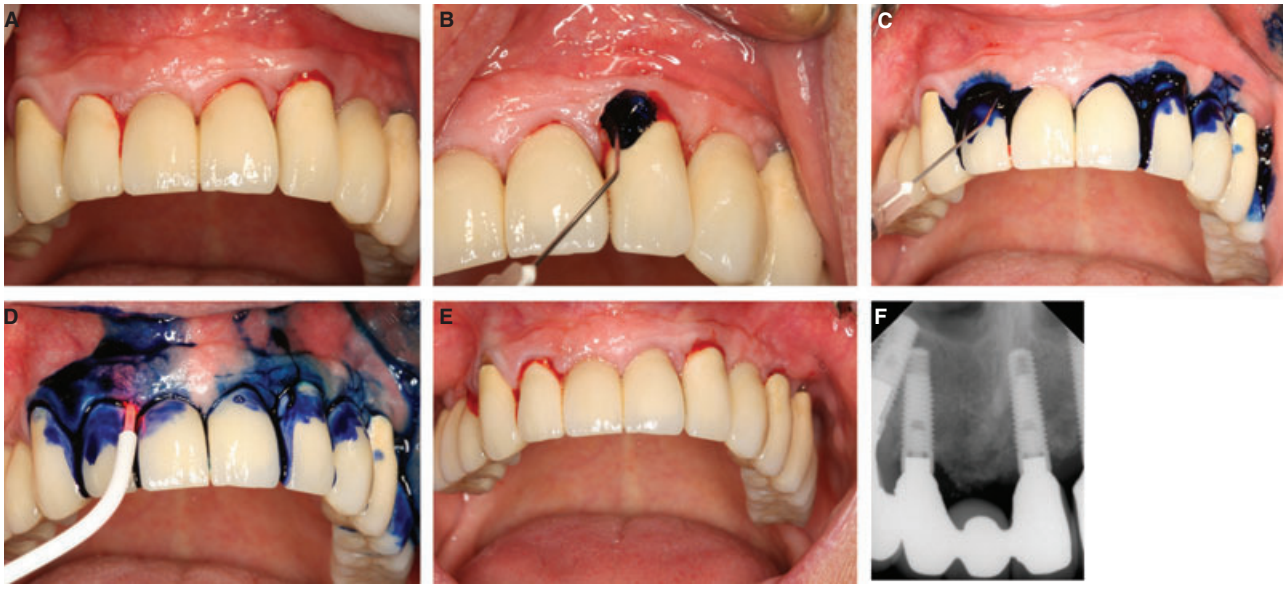

Fig. 4. Clinical application of antimicrobial photodynamic therapy in the treatment of peri-implantitis (A) The clinical situation before nonsurgical peri-implant therapy and antimicrobial photodynamic therapy of a 32-year-old patient. The clinical parameters at implant #22 were a probing pocket depth of 5 mm and relative attachment level of 5 mm with bleeding on probing. (B) Application of the photosensitizer. The photosensitizer applied was a 'Phenothiazine Chloride' (HELBO® Blue Photosensitizer, HELBO® Photodynamic Systems GmbH & Co. KG, Grieskirchen, Austria). The photosensitizer was placed in the peri-implant pocket for 3 mins. (C) After application of the photosensitizer. (D) Irradiation with the diode laser. Laser irradiation was performed with a diode laser of 670 nm wavelength at 75 mW of power output (HELBO® TheraLite Laser, HELBO® Photodynamic Systems GmbH & Co. KG, Grieskirchen, Austria). Laser irradiation was performed for 1 min. (E) The clinical situation 6 months after therapy. The treated site showed limited clinical improvement with the peri-implant pocket remaining and bleeding on probing occurring after therapy. Nonsurgical treatment of a peri-implant pocket using antimicrobial photodynamic therapy monotherapy did not improve the treated site. (F) Radiograph of the treated implant before treatment. (Operator: F.S.)

blue O alone. None of the smears obtained from the plates subjected to photodynamic therapy showed bacterial growth of any of the microorganisms, while in the other treatment groups all three species of bacteria were detected after treatment. Scanning electron microscopic analysis showed that antimicrobial photodynamic therapy led to bacterial cell destruction without damage to the titanium surface.

In an animal study using dogs, Hayek et al. (49) compared the effects of antimicrobial photodynamic therapy (paste-based Azulene + 50 mW diode laser) with those of a conventional technique, which included mucoperiosteal flap surgery and irrigation with chlorhexidine, on microbial reduction following ligature-induced peri-implantitis. Periodontal pathogens, such as *Prevotella* ssp., *Fusobacterium* ssp. and *Streptococcus beta-haemolyticus*, were effectively reduced by photodynamic therapy to a level equivalent to that achieved by conventional treatment. The authors emphasized the favorable application of the photosensitizer in a paste base instead of in liquid solution, which allows it to be removed easily after treatment without any compromise in esthetics. Similar antimicrobial results were also obtained by Shibli et al. (113), who reported that antimicrobial photodynamic therapy (toluidine blue O + 50 mW diode laser) could reduce the bacterial count of *P. intermedia*, *P. nigrescens*, *Fusobacterium* spp. and beta-hemolytic *Streptococcus* in ligature-induced peri-implantitis of dogs and, in some samples, complete elimination of those bacteria could be obtained. In another study, Shibli et al. (112) evaluated the efficacy of antimicrobial photodynamic therapy associated with guided bone regeneration for the treatment of ligature-induced peri-implantitis in dogs, using implants with different surface characteristics. They reported that antimicrobial photodynamic therapy may be effectively applied for decontamination of implant surfaces and that bone defect fill and re-osseointegration could be achieved by its combination with guided bone regeneration. Later, Shibli et al. (114) compared the effects of the combination of antimicrobial photodynamic therapy and guided bone regeneration with those of conventional mechanical debridement associated with guided bone regeneration in the treatment of

Table 6. Studies on the application of photodynamic therapy in the treatment of peri-implantitis

Author and year (reference)	Type of study	Light (wavelength)	Photosensitizer (concentration)	Light parameters and time of exposure	Purpose of application	Findings
Haas et al. 1997 (46)	*In vitro* (titanium plates)	Diode laser (905 nm)	TBO (100 μg/ml)	CW, total power of 7.3 mW 60 s each plate	Investigate the microbiological effects of PDT against *A.a.*, *P.g.* and *P.i.* adhered to titanium plates	No bacterial growth of any of the microorganisms on the smear taken from the plates treated with PDT, in contrast to that of the nontreated plates in which all bacteria were detected
Shibli et al. 2003 (112)	*In vivo* (six dogs)	Diode laser (685 nm)	TBO (100 μg/ml)	CW, 50 mW 80 s per implant	Disinfect the contaminated implant surface in the treatment of ligature-induced peri-implantitis	PDT may be effectively applied for decontamination of the implant surface. Histologically, bone defect fill and re-osseointegration could be achieved by its combination with GBR at 5 months postsurgery
Shibli et al. 2003 (113)	*In vivo* (six dogs)	Diode laser (685 nm)	TBO (100 μg/ml)	CW, 50 mW 80 s per implant	Examine the microbiological effects of PDT against *A.a.*, *P.g.* and *P.i.* on the surface of implants affected by ligature-induced peri-implantitis	PDT reduced the bacterial count of *P.i.*, *P.n.*, *Fusobacterium spp.* and *beta-hemolytic Streptococcus* on the implant surface
Hayek et al. 2005 (49)	*In vivo* (nine dogs)	Diode laser (660 nm)	Azulene 25% (w/v)	CW, 40 mW 180 s per implant	Examine the microbiological effects of PDT on implants affected by ligature-induced peri-implantitis	PDT reduced the bacterial count of *Prevotella* sp., *Fusobacterium* sp. and *S. Beta-haemolyticus* on the implant surface
Shibli et al. 2006 (114)	*In vivo* (five dogs)	Diode laser (830 nm)	TBO (100 μg/ml)	CW, 50 mW 80 s per implant	Disinfect the contaminated implant surface in the treatment of ligature-induced peri-implantitis	The combination of PDT and GBR produced higher bone gain compared with the combination of mechanical debridement and GBR at 5 months postsurgery

Table 6. *Continued*

Author and year (reference)	Type of study	Light (wavelength)	Photosensitizer (concentration)	Light parameters and time of exposure	Purpose of application	Findings
Haas et al. 2000 (47)	Clinical study, case series (17 subjects)	Diode laser (906 nm)	TBO* (100 μg / ml)	CW, NA 120 s per implant	Disinfect the contaminated implant surface in the treatment of peri-implantitis	Combination of PDT, autogenous bone grafts and membrane placement could reduce bone defects
Dörtbudak et al. 2001 (37)	Clinical study, case series (15 subjects)	Diode laser (690 nm)	TBO (100 μg / ml)	CW, NA 60 s per implant	Examine the microbiological effects of PDT against *A.a.*, *P.g.* and *P.i.* on the surface of implants affected by peri-implantitis	PDT significantly decreased all bacterial counts, but TBO alone could also reduce the bacterial counts to some extent

CW, continuous wave; GBR, guided bone regeneration; NA, not available; PDT, photodynamic therapy; TBO, toluidine blue O; w / v, weight / volume; *A.a.*, *Aggregatibacter actinomycetemcomitans*; *P.g.*, *Porphyromonas gingivalis*; *P.i.*, *Prevotella intermedia*; *P.n.*, *Prevotella nigrescens*.
*Toluidin-blau O Zinkchlorid Dopplersalz, Merck KGaA, Vienna, Austria.

ligature-induced peri-implantitis in dogs. They showed that the combination of antimicrobial photodynamic therapy and guided bone regeneration resulted in greater bone gain than conventional mechanical debridement associated with guided bone regeneration, which was independent of the characteristics of the implant surface and could achieve significant bone gain: the mean percentage of re-osseointegration of implant surfaces ranged from 31 to 41% for the photodynamic therapy group and from 0 to 14% for the control group at 5 months postsurgery.

In a clinical case-series study, Haas et al. (47) investigated the clinical effects of treatment of antimicrobial photodynamic therapy (toludine blue O + diode laser) in combination with guided bone regeneration using autogenous bone grafts on 24 implants diagnosed with peri-implantitis in 17 patients. They reported that 21 implants out of 24 showed improvements in the bone defect after a mean observation period of 9.5 months. Dörtbudak et al. (37) examined the effectiveness of antimicrobial photodynamic therapy in treating contaminated implant surfaces by evaluating the remaining levels of *A. actinomycetemcomitans*, *P. gingivalis* and *P. intermedia*. Microbiological samples on 15 patients diagnosed with peri-implantitis were taken from the same implants before, after application of toluidine blue O alone and after the application of photodynamic therapy. Significant decreases of all species of bacteria were observed following photodynamic therapy by comparison with baseline levels. However, the application of toluidine blue O alone without laser light also resulted in a decrease of all bacterial species, and complete bacterial reduction was not achieved with either the application of toluidine blue O alone or of photodynamic therapy alone. Furthermore, in a case report Schuckert et al. (104) demonstrated effective bone regeneration within bone defects around implants affected by peri-implantitis following surgical therapy using photodynamic therapy (tolonium chlorine + 100 mW diode laser) to decontaminate the implant surface and the application of recombinant human bone morphogenetic protein-2.

Thus, the results of the previous studies indicate that the application of antimicrobial photodynamic therapy can effectively reduce the prevalence of pathogens on implant surfaces without causing any side effects on the implant and bone surfaces. However, *in vivo* and clinical studies are very limited and significant clinical effects of antimicrobial photodynamic therapy have not yet been demon-

strated. From our limited clinical experience, adjunctive application of antimicrobial photodynamic therapy during nonsurgical treatment of peri-
46 implantitis did not provide significant clinical improvements. Therefore, further animal and clinical studies to establish the optimal conditions and procedures for antimicrobial photodynamic therapy in the nonsurgical or surgical treatment of peri-implantitis, and to demonstrate the advantages of antimicrobial photodynamic therapy over conventional chemical methods for implant surface decontamination, should be encouraged.

Risks and side effects of antimicrobial photodynamic therapy

A critical issue when applying novel techniques relates to their clinical safety. The risks and side effects of antimicrobial photodynamic therapy are basically classified into two categories: one relates to the effect of light energy itself; and the other is related to the photosensitizer and the photochemical reaction (lethal photosensitization).

Regarding the light source itself, when using lasers as a matter of course there are some rules and concerns that should be kept in mind during clinical application. First, potential inadvertent irradiation of the patient's eyes must be strictly avoided during treatment, even though the laser power employed is very low (1). The most important precaution in laser surgery is the use of protective glasses by the patient, the operator and the assistants (1). Even in the case of nonlaser light, the wearing of eye glasses is recommended during the use of relatively high-intensity light. Second, during treatment with high-level lasers, thermogenesis occurring as a result of the interaction of the laser with the tissues must be addressed and well controlled. However, the wavelengths of diode lasers exhibiting deep-tissue penetration basically do not interact with the periodontal tissues within the pocket or tooth crown. Therefore, photodynamic therapy as a low-level therapy, using a diode laser with a short irradiation time, is considered not to produce any thermal changes within the gingival tissues and root surfaces, or destruction of the intact attachment apparatus at the base of pockets. Furthermore, the liquid of the photosensitizer solution applied may minimize thermal generation within the pockets. However, an extended
45 period of irradiation at the same spot must be avoided to prevent any thermal accumulation or injury to the deeper tissues, such as bone or dental pulp. Thus, in order for lasers to be used safely within the clinical
46 environment, the practitioner should have precise knowledge of the characteristics and effects of the laser system and its performance during application, and should exercise appropriate caution during use.

With respect to the photosensitizers and photochemical reactions, it is important to know if the targeted bacteria can be killed by the application of antimicrobial photodynamic therapy without the occurrence of any adverse effects in the surrounding periodontal tissues. Although the safety of antimicrobial photodynamic therapy in the host periodontal tissues has been demonstrated by several animal and clinical studies (6, 27, 66), there is still concern regarding short-term and long-term changes of biological tissues, including the periodontium, when antimicrobial photodynamic therapy is applied adjunctively to conventional mechanical therapy. Research performed *in vitro* and in animal models suggests that the adverse effects on host tissues may not be a problem because the photosensitizer concentrations and light energy doses necessary to kill the infecting microorganism have little effect on adjacent host tissues (66, 92, 120). Safe application of photodynamic therapy in the treatment of oral infections without damaging adjacent normal tissues, such as the tongue (92) or the buccal mucosa (57), has been previously demonstrated. Additionally, Luan et al. (66) reported that no necrotic or inflammatory changes were found in periodontal tissues following photodynamic therapy treatment, suggesting that antimicrobial photodynamic therapy is a safe therapy that does not damage the adjacent normal tissues.

Nevertheless, it should be pointed out that the photosensitizer alone can exhibit bactericidal action (37). The photosensitizer may be toxic to some extent and the effect on the periodontal tissues and cells should be precisely clarified. Also, most of the dyes adhere strongly to the soft tissue surface of the pocket, and retention of the dyes in the pocket, even for a short period of time, may affect periodontal tissue attachment during wound healing. It seems that removal of the dye solution has not been routinely performed clinically after photosensitization procedures. Further studies should be performed to investigate the longevity and the effects of remaining dyes and the necessity for an efficient technique to remove the dye solutions from the periodontal pockets. In addition, the use of photosensitizers can compromise the patients' esthetics by producing temporary pigmentation of the periodontal tissues.

Thus, the use of photosensitizers with a paste base instead of liquids has been suggested, because pastes can be easily removed following treatment (49).

In addition, it still remains to be clarified whether selective killing of periodontal pathogens by antimicrobial photodynamic therapy really occurs without affecting the normal oral microflora. A recent study has shown that, in the treatment of infections, a specific bacterium can be targeted and killed using photosensitizers conjugated to specific antibodies (16), thus without affecting the host's normal microbial flora. Further studies are necessary to develop and improve the current photosensitizers in order to assure safety and to optimize efficiency.

Current status of antimicrobial photodynamic therapy and future directions

Antimicrobial photodynamic chemotherapy seems to be an attractive option as a low-cost treatment approach in the field of periodontics and implant dentistry. Because antimicrobial photodynamic therapy can be applied locally, the systemic administration of antibiotics can be avoided in the treatment of localized infections. In antimicrobial photodynamic therapy, a high concentration of the chemical agent at the locus of infection enables efficient bacterial elimination without inducing side effects on the host tissue (66, 115).

Although the available data from *in vitro* and *in vivo* studies has shown that antimicrobial photodynamic therapy has a high bactericidal effect against periodontal pathogens, it has not been clarified which photosensitizer and light source would provide the most suitable combination to obtain the desired bactericidal effect in the clinical situation. Toluidine blue O and methylene blue are the most commonly used
47 photosensitizers, and the diode laser is the main light source applied in antimicrobial photodynamic therapy. However, it is still unclear which is more important in antimicrobial photodynamic therapy – the light
48 source for activation or the type of photosensitizer. Moreover, the optimal time of photosensitizer application, as well as the time of light exposure required in order to achieve the desired optimal result, are unknown.

Regarding clinical application, whilst the manufacturer recommends that antimicrobial photodynamic therapy treatment should be performed repeatedly during the first weeks of healing to enhance the antimicrobial effect, the application of photodynamic therapy has been mainly performed in a single episode in the aforementioned clinical studies. Multiple courses of photodynamic therapy may improve healing outcomes and its long-term effects. However, it has not been established how often photodynamic therapy should be applied for the effective elimination of bacteria, as well as prevention of recolonization by the bacteria of sites previously treated by nonsurgical periodontal therapy. Future studies are needed to elucidate if multiple courses of antimicrobial photodynamic therapy may enhance treatment outcomes.

Based on the current data from randomized controlled clinical studies, adjunctive use of antimicrobial photodynamic therapy during nonsurgical periodontal therapy may lead to improved clinical results. However, any definitive conclusion regarding the advantages of the adjunctive application of antimicrobial photodynamic therapy in the treatment of periodontitis and peri-implantitis may not yet be warranted because there are only a limited number of clinical studies showing significantly better clinical and microbiological improvements with antimicrobial photodynamic therapy adjunctive to mechanical debridement compared with mechanical debridement alone. There is no study comparing the antimicrobial effects of adjunctive applications of antimicrobial photodynamic therapy with that of local / and or systemic antibiotics following mechanical debridement. Thus, it is unclear whether antimicrobial photodynamic monotherapy could be used as an alternative to systemic or local antibiotics in patients with aggressive or severe chronic periodontitis. Moreover, it has not been demonstrated whether antimicrobial photodynamic therapy can completely eliminate some putative periodontal pathogens, such as *A. actinomycetemcomitans* or *P. gingivalis*, *in vivo* from human subjects with periodontitis. Antimicrobial photodynamic therapy might be an alternative to nonsurgical periodontal mechanical therapy in periodontal sites with no subgingival calculus deposition on the root surface. However, there are insufficient current clinical data to support this idea. Also, clinical and microbiological studies comparing the effects of adjunctive application of photodynamic therapy with mechanical therapy, and studies of the high-level laser treatment applied adjunctively or as an alternative to conventional mechanical therapy, are necessary.

If all the questions described above are answered and the advantages of photodynamic therapy are clarified, antimicrobial photodynamic therapy could become widely applied in clinical practice in the

near future and may become a reliable choice in the antimicrobial approach for the treatment of periodontitis. In periodontal therapy, antimicrobial photodynamic therapy would be employed adjunctively to conventional mechanical treatment to treat moderate to severe periodontal pockets during the initial nonsurgical or surgical therapy, or as supportive therapy of the remaining pockets during the maintenance period. Regarding peri-implantitis, application of antimicrobial photodynamic therapy may be indicated as an adjunct following debridement in surgical therapy. Antimicrobial photodynamic monotherapy does not appear to be promising in nonsurgical peri-implant therapy because of the lack of effective tools for implant surface debridement when there is no direct view of the treated site.

The use of low-level energy lasers (i.e. diode lasers) is reported to exert additional positive effects on the surrounding tissues and cells, and they may further contribute favorably to the healing of periodontal tissues as a result of the potential biomodulatory effects, such as stimulation and proliferation of cells (60). When using lasers in antimicrobial photodynamic therapy, not only bactericidal effects, but also the additional photo-biomodulatory effects, might be expected and utilized to achieve improved clinical results (9).

An alternative use for antimicrobial photodynamic therapy may be to aid in mechanical plaque control and to attain a high-level eradication of bacteria from the oral cavity. A 'photobrush' system can be created by the combination of a brush that emits a harmless light-emitting diode or a low-level laser light and toothpaste that includes the appropriate photosensitizer. The periodical usage of the 'photobrush' at home or within the dental surgery to achieve high levels of plaque control might prevent the development or progression of periodontal and peri-implant diseases in the near future (9).

Conclusions

Antimicrobial photodynamic therapy seems to be a unique and interesting therapeutic approach towards the treatment of periodontitis and peri-implantitis. The results of a number of *in vitro* studies clearly demonstrate the effective and efficient bactericidal effect of antimicrobial photodynamic therapy. However, sufficient clinical and microbiological data that support the superior effects of the adjunctive use of photodynamic therapy have not been demonstrated *in vivo* or clinically in either periodontal or peri-implant therapies. The discrepancy in the results obtained from previous clinical studies may be a result of the differences in treatment conditions and parameters. Therefore, further *in vivo* and clinical studies are necessary to determine the optimal conditions of this novel therapy. Also, further randomized long-term clinical studies and meta-analyses are necessary to demonstrate the beneficial effects of antimicrobial photochemical therapy and their real advantages in comparison with conventional methods. Antimicrobial photodynamic therapy may hold promise as a substitute for currently available chemotherapy in the treatment of periodontal and peri-implant diseases.

References

1. AAP. The Research, Science and Therapy Committee of the American Academy of Periodontology. Lasers in periodontics (Academy report), authored by Cohen RE and Ammons WF, revised by Rossman JA. *J Periodontol* 2002: **73**: 1231–1239.
2. Adriaens PA, Adriaens LM. Effects of nonsurgical periodontal therapy on hard and soft tissues. *Periodontol 2000* 2004: **36**: 121–145.
3. Aghahosseini F, Arbabi-Kalati F, Fashtami LA, Djavid GE, Fateh M, Beitollahi JM. Methylene blue-mediated photodynamic therapy: a possible alternative treatment for oral lichen planus. *Lasers Surg Med* 2006: **38**: 33–38.
4. Allison RR, Bagnato VS, Cuenca R, Downie GH, Sibata CH. The future of photodynamic therapy in oncology. *Future Oncol* 2006: **2**: 53–71.
5. Amano A. Disruption of epithelial barrier and impairment of cellular function by Porphyromonas gingivalis. *Front Biosci* 2007: **12**: 3965–3974.
6. Andersen R, Loebel N, Hammond D, Wilson M. Treatment of periodontal disease by photodisinfection compared to scaling and root planing. *J Clin Dent* 2007: **18**: 34–38.
7. Ando Y, Aoki A, Watanabe H, Ishikawa I. Bactericidal effect of erbium YAG laser on periodontopathic bacteria. *Lasers Surg Med* 1996: **19**: 190–200.
8. Aoki A, Sasaki KM, Watanabe H, Ishikawa I. Lasers in nonsurgical periodontal therapy. *Periodontol 2000* 2004: **36**: 59–97.
9. Aoki A, Takasaki AA, Pourzarandian A, Mizutani K, Ruwanpura SM, Iwasaki K, Noguchi K, Oda S, Watanabe H, Ishikawa I, Izumi Y. Photo-bio-modulation Laser Strategies in Periodontal Therapy. In: Waynant R, Tata DB editor. *Proceedings of light-activated tissue regeneration and therapy II*. Tomar, Portugal: Springer, 2007: 181–190.
10. Aoki A, Mizutani K, Takasaki AA, Sasaki KM, Nagai S, Schwarz F, Yoshida I, Eguro T, Zeredo JL, Izumi Y. Current status of clinical laser applications in periodontal therapy. *Gen Dent* 2009 (in press).
11. Augthun M, Tinschert J, Huber A. *In vitro* studies on the effect of cleaning methods on different implant surfaces. *J Periodontol* 1998: **69**: 857–864.

12. Bertoloni G, Rossi F, Valduga G, Jori G, van Lier J. Photosensitizing activity of water- and lipid-soluble phthalocyanines on *Escherichia coli*. *FEMS Microbiol Lett* 1990: **59**: 149–155.
13. Bertoloni G, Lauro FM, Cortella G, Merchat M. Photosensitizing activity of hematoporphyrin on *Staphylococcus aureus* cells. *Biochim Biophys Acta* 2000: **1475**: 169–174.
14. Bhatti M, MacRobert A, 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.
15. Bhatti M, MacRobert A, Meghji S, Henderson B, Wilson M. A study of the uptake of toluidine blue O by *Porphyromonas gingivalis* and the mechanism of lethal photosensitization. *Photochem Photobiol* 1998: **68**: 370–376.
16. Bhatti M, MacRobert A, Henderson B, Shepherd P, Cridland J, Wilson M. Antibody-targeted lethal photosensitization of *Porphyromonas gingivalis*. *Antimicrob Agents Chemother* 2000: **44**: 2615–2618.
17. Bhatti M, MacRobert A, Henderson B, Wilson M. Exposure of *Porphyromonas gingivalis* to red light in the presence of the light-activated antimicrobial agent toluidine blue decreases membrane fluidity. *Curr Microbiol* 2002: **45**: 118–122.
18. Bonsor S, Nichol R, Reid T, Pearson G. Microbiological evaluation of photo-activated disinfection in endodontics (an *in vivo* study). *Br Dent J* 2006: **200**: 337–341, discussion 329.
19. Bonsor S, Pearson G. Current clinical applications of photo-activated disinfection in restorative dentistry. *Dent Update* 2006: **150**: 153.
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. Brink B, Romanos GE. Mikrobiologische Untersuchungen bei adjuvanten Laseranwendungen in der Parodontologie. *Laser Zahnheilkunde* 2007: **2**: 37–42 (in German).
22. Brink B, Romanos GE. Laseranwendung in der Parodontaltherapie. Klinische Daten einer Untersuchung aus der freien Praxis. *Laser Zahnheilkunde* 2007: **3**: 165–171 (in German).
23. Busetti A, Soncin M, Jori G, Kenney ME, Rodgers MA. Treatment of malignant melanoma by high-peak-power 1064 nm irradiation followed by photodynamic therapy. *Photochem Photobiol* 1998: **68**: 377–381.
24. Buytaert E, Dewaele M, Agostinis P. Molecular effectors of multiple cell death pathways initiated by photodynamic therapy. *Biochim Biophys Acta* 2007: **1776**: 86–107.
25. Chan Y, Lai CH. Bactericidal effects of different laser wavelengths on periodontopathic germs in photodynamic therapy. *Lasers Med Sci* 2003: **18**: 51–55.
26. Chondros P, Nikolidakis D, Christodoulides N, Rossler R, Gutknecht N, Sculean A. Photodynamic therapy as adjunct to non-surgical periodontal treatment in patients on periodontal maintenance: a randomized controlled clinical trial. *Lasers Med Sci* 2008 (in press).
27. Christodoulides N, Nikolidakis D, Chondros P, Becker J, Schwarz F, Rossler R, Sculean A. Photodynamic therapy as an adjunct to non-surgical peridoontal treatment: a randomized controlled clinical trial. *J Periodontol* 2008: **79**: 1638–1644.
28. Cobb CM, McCawley TK, Killoy WJ. A preliminary study on the effects of the Nd:YAG laser on root surfaces and subgingival microflora *in vivo*. *J Periodontol* 1992: **63**: 701–707.
29. Cobb CM. Lasers in periodontics: a review of the literature. *J Periodontol* 2006: **77**: 545–564.
30. Darveau RP, Tanner A, Page RC. The microbial challenge in periodontitis. *Periodontol 2000* 1997: **14**: 12–32.
31. de 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.
32. de Almeida JM, Theodoro LH, Bosco AF, Nagata MJ, Oshiiwa M, Garcia VG. *In vivo* effect of photodynamic therapy on periodontal bone loss in dental furcations. *J Periodontol* 2008: **79**: 1081–1088.
33. de Almeida JM, Theodoro LH, Bosco AF, Nagata MJH, Bonfante S, Garcia VG. Treatment of experimental periodontal disease by photodynamic therapy in diabetes. *J Periodontol* 2008 (in press).
34. de Oliveira RR, Schwartz-Filho HO, Novaes AB Jr, Taba M Jr. Antimicrobial photodynamic therapy in the non-surgical treatment of aggressive periodontitis: a preliminary randomized controlled clinical study. *J Periodontol* 2007: **78**: 965–973.
35. Dobson J, Wilson M. Sensitization of oral bacteria in biofilms to killing by light from a low-power laser. *Arch Oral Biol* 1992: **37**: 883–887.
36. Donnelly RF, McCarron PA, Tunney MM, David Woolfson A. Potential of photodynamic therapy in treatment of fungal infections of the mouth. Design and characterisation of a mucoadhesive patch containing toluidine blue O. *J Photochem Photobiol B* 2007: **86**: 59–69.
37. Dortbudak 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.
38. Dougherty TJ, Gomer CJ, Henderson BW, Jori G, Kessel D, Korbelik M, Moan J, Peng Q. Photodynamic therapy. *J Natl Cancer Inst* 1998: **90**: 889–905.
39. Drisko CH. Nonsurgical periodontal therapy. *Periodontol 2000* 2001: **25**: 77–88.
40. Fan KF, Hopper C, Speight PM, Buonaccorsi G, MacRobert AJ, Bown SG. Photodynamic therapy using 5-aminolevulinic acid for premalignant and malignant lesions of the oral cavity. *Cancer* 1996: **78**: 1374–1383.
41. Fiel RJ, Datta-Gupta N, Mark EH, Howard JC. Induction of DNA damage by porphyrin photosensitizers. *Cancer Res* 1981: **41**: 3543–3545.
42. Fimple JL, Fontana CR, Foschi F, Ruggiero K, Song X, Pagonis TC, Tanner AC, Kent R, Doukas AG, Stashenko PP, Soukos NS. Photodynamic treatment of endodontic polymicrobial infection *in vitro*. *J Endod* 2008: **34**: 728–734.
43. Foote CS. Definition of type I and type II photosensitized oxidation. *Photochem Photobiol* 1991: **54**: 659.
44. Garcez AS, Ribeiro MS, Tegos GP, Nunez SC, Jorge AO, Hamblin MR. Antimicrobial photodynamic therapy combined with conventional endodontic treatment to eliminate root canal biofilm infection. *Lasers Surg Med* 2007: **39**: 59–66.
45. Garcez AS, Nunez SC, Hamblin MR, Ribeiro MS. Antimicrobial effects of photodynamic therapy on patients with

necrotic pulps and periapical lesion. *J Endod* 2008: **34**: 138–142.
46. Haas R, Dortbudak 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.
47. Haas R, Baron M, Dortbudak O, Watzek G. Lethal photosensitization, autogenous bone, and e-PTFE membrane for the treatment of peri-implantitis: preliminary results. *Int J Oral Maxillofac Implants* 2000: **15**: 374–382.
48. Hamblin MR, Hasan T. Photodynamic therapy: a new antimicrobial approach to infectious disease? *Photochem Photobiol Sci* 2004: **3**: 436–450.
49. Hayek RR, Araujo 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.
50. Henry CA, Judy M, Dyer B, Wagner M, Matthews JL. Sensitivity of Porphyromonas and Prevotella species in liquid media to argon laser. *Photochem Photobiol* 1995: **61**: 410–413.
51. Henry CA, Dyer B, Wagner M, Judy M, Matthews JL. Phototoxicity of argon laser irradiation on biofilms of Porphyromonas and Prevotella species. *J Photochem Photobiol B* 1996: **34**: 123–128.
52. Ishikawa I, Aoki A, Takasaki AA. Potential applications of Erbium:YAG laser in periodontics. *J Periodontal Res* 2004: **39**: 275–285.
53. Ishikawa I, Aoki A, Takasaki AA, Mizutani K, Sasaki KM, Izumi Y. Application of lasers in periodontics – True
55 Innovation or Myth? *Periodontol 2000* 2009 (in press).
54. Karring ES, Stavropoulos A, Ellegaard B, Karring T. Treatment of peri-implantitis by the Vector system. *Clin Oral Implants Res* 2005: **16**: 288–293.
55. Kennedy JC, Pottier RH, Pross DC. Photodynamic therapy with endogenous protoporphyrin IX: basic principles and present clinical experience. *J Photochem Photobiol B* 1990: **6**: 143–148.
56. Komerik N, Wilson M, Poole S. The effect of photodynamic action on two virulence factors of gram-negative bacteria. *Photochem Photobiol* 2000: **72**: 676–680.
57. Komerik N, Curnow A, MacRobert AJ, Hopper C, Speight PM, Wilson M. Fluorescence biodistribution and photosensitising activity of toluidine blue o on rat buccal mucosa. *Lasers Med Sci* 2002: **17**: 86–92.
58. Komerik 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.
59. Konopka K, Goslinski T. Photodynamic therapy in dentistry. *J Dent Res* 2007: **86**: 694–707.
60. 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.
61. Kreisler M, Gotz H, Duschner H. Effect of Nd:YAG, Ho:YAG, Er:YAG, CO_2, and GaAIAs laser irradiation on surface properties of endosseous dental implants. *Int J Oral Maxillofac Implants* 2002: **17**: 202–211.
62. Kreisler M, Kohnen W, Marinello C, Gotz H, Duschner H, Jansen B, d'Hoedt B. Bactericidal effect of the Er:YAG laser on dental implant surfaces: an *in vitro* study. *J Periodontol* 2002: **73**: 1292–1298.
63. Kubler A, Haase T, Rheinwald M, Barth T, Muhling J. Treatment of oral leukoplakia by topical application of 5-aminolevulinic acid. *Int J Oral Maxillofac Surg* 1998: **27**: 466–469.
64. Lambrecht B, Mohr H, Knuver-Hopf J, Schmitt H. Photoinactivation of viruses in human fresh plasma by phenothiazine dyes in combination with visible light. *Vox Sang* 1991: **60**: 207–213.
65. Listgarten MA, Lai CH. Comparative microbiological characteristics of failing implants and periodontally diseased teeth. *J Periodontol* 1999: **70**: 431–437.
66. Luan XL, Qin YL, Bi LJ, Hu CY, Zhang ZG, Lin J, Zhou CN. Histological evaluation of the safety of toluidine blue-mediated photosensitization to periodontal tissues in
56 mice. *Lasers Med Sci* 2008.
67. Lundgren J, Olofsson J, Hellquist H. Toluidine blue. An aid in the microlaryngoscopic diagnosis of glottic lesions? *Arch Otolaryngol* 1979: **105**: 169–174.
68. Magnusson I, Low SB, McArthur WP, Marks RG, Walker CB, Maruniak J, Taylor M, Padgett P, Jung J, Clark WB. Treatment of subjects with refractory periodontal disease. *J Clin Periodontol* 1994: **21**: 628–637.
69. Maisch T, Szeimies RM, Jori G, Abels C. Antibacterial photodynamic therapy in dermatology. *Photochem Photobiol Sci* 2004: **3**: 907–917.
70. Maisch T. Anti-microbial photodynamic therapy: useful in the future? *Lasers Med Sci* 2007: **22**: 83–91.
71. Malik Z, Ladan H, Nitzan Y. Photodynamic inactivation of Gram-negative bacteria: problems and possible solutions. *J Photochem Photobiol B* 1992: **14**: 262–266.
72. Matevski D, Weersink R, Tenenbaum HC, Wilson B, Ellen RP, Lepine G. Lethal photosensitization of periodontal pathogens by a red-filtered Xenon lamp *in vitro*. *J Periodontal Res* 2003: **38**: 428–435.
73. Meisel P, Kocher T. Photodynamic therapy for periodontal diseases: state of the art. *J Photochem Photobiol B* 2005: **79**: 159–170.
74. Merchat M, Bertolini G, Giacomini P, Villanueva A, Jori G. Meso-substituted cationic porphyrins as efficient photosensitizers of gram-positive and gram-negative bacteria. *J Photochem Photobiol B* 1996: **32**: 153–157.
75. Merchat M, Spikes JD, Bertoloni G, Jori G. Studies on the mechanism of bacteria photosensitization by meso-substituted cationic porphyrins. *J Photochem Photobiol B* 1996: **35**: 149–157.
76. Meyer DH, Sreenivasan PK, Fives-Taylor PM. Evidence for invasion of a human oral cell line by *Actinobacillus actinomycetemcomitans*. *Infect Immun* 1991: **59**: 2719–2726.
77. Millson CE, Wilson M, Macrobert AJ, Bedwell J, Bown SG. The killing of Helicobacter pylori by low-power laser light in the presence of a photosensitiser. *J Med Microbiol* 1996: **44**: 245–252.
78. Moan J, Berg K. The photodegradation of porphyrins in cells can be used to estimate the lifetime of singlet oxygen. *Photochem Photobiol* 1991: **53**: 549–553.
79. Mohr H, Lambrecht B, Schmitt H. Photo-inactivation of viruses in therapeutical plasma. *Dev Biol Stand* 1993: **81**: 177–183.

80. Mombelli A, van Oosten MA, Schurch E Jr, Land NP. The microbiota associated with successful or failing osseointegrated titanium implants. *Oral Microbiol Immunol* 1987: **2**: 145–151.
81. Mombelli A, Lang NP. Antimicrobial treatment of peri-implant infections. *Clin Oral Implants Res* 1992: **3**: 162–168.
82. Moritz A, Gutknecht N, Doertbudak O, Goharkhay K, Schoop U, Schauer P, Sperr W. Bacterial reduction in periodontal pockets through irradiation with a diode laser: a pilot study. *J Clin Laser Med Surg* 1997: **15**: 33–37.
83. Morlock BJ, Pippin DJ, Cobb CM, Killoy WJ, Rapley JW. The effect of Nd:YAG laser exposure on root surfaces when used as an adjunct to root planing: an *in vitro* study. *J Periodontol* 1992: **63**: 637–641.
84. Mouhyi J, Sennerby L, Nammour S, Guillaume P, Van Reck J. Temperature increases during surface decontamination of titanium implants using CO_2 laser. *Clin Oral Implants Res* 1999: **10**: 54–61.
85. Nikaido H. Prevention of drug access to bacterial targets: permeability barriers and active efflux. *Science* 1994: **264**: 382–388.
86. Ochsner M. Photophysical and photobiological processes in the photodynamic therapy of tumours. *J Photochem Photobiol B* 1997: **39**: 1–18.
87. Ojetti V, Persiani R, Nista EC, Rausei S, Lecca G, Migneco A, Cananzi FC, Cammarota G, D'Ugo D, Gasbarrini G, Gasbarrini A. A case-control study comparing methylene blue directed biopsies and random biopsies for detecting pre-cancerous lesions in the follow-up of gastric cancer patients. *Eur Rev Med Pharmacol Sci* 2007: **11**: 291–296.
88. O'Neill JF, Hope CK, Wilson M. Oral bacteria in multispecies biofilms can be killed by red light in the presence of toluidine blue. *Lasers Surg Med* 2002: **31**: 86–90.
89. Oyster DK, Parker WB, Gher ME. CO_2 lasers and temperature changes of titanium implants. *J Periodontol* 1995: **66**: 1017–1024.
90. Panjehpour M, Overholt BF, DeNovo RC, Petersen MG, Sneed RE. Comparative study between pulsed and continuous wave lasers for Photofrin photodynamic therapy. *Lasers Surg Med* 1993: **13**: 296–304.
91. Paulino T, Ribeiro K, Thedei GJ, Tedesco A, Ciancaglini P. Use of hand held photopolymerizer to photoinactivate *Streptococcus mutans*. *Arch Oral Biol* 2005: **50**: 353–359.
92. Pe MB, Sano K, Inokuchi T. Effects of photodynamic therapy in the normal mouse tongue. *J Oral Maxillofac Surg* 1993: **51**: 1129–1134.
93. Peng Q, Moan J, Nesland JM. Correlation of subcellular and intratumoral photosensitizer localization with ultrastructural features after photodynamic therapy. *Ultrastruct Pathol* 1996: **20**: 109–129.
94. Qadri T, Miranda L, Tuner J, Gustafsson A. The short-term effects of low-level lasers as adjunct therapy in the treatment of periodontal inflammation. *J Clin Periodontol* 2005: **32**: 714–719.
95. Qin Y, Luan X, Bi L, He G, Bai X, Zhou C, Zhang Z. Toluidine blue-mediated photoinactivation of periodontal pathogens from supragingival plaques. *Lasers Med Sci* 2008: **23**: 49–54.
96. 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.
97. Quirynen M, Teughels W, De Soete M, van Steenberghe D. Topical antiseptics and antibiotics in the initial therapy of chronic adult periodontitis: microbiological aspects. *Periodontol 2000* 2002: **28**: 72–90.
98. Radvar M, MacFarlane TW, MacKenzie D, Whitters CJ, Payne AP, Kinane DF. An evaluation of the Nd:YAG laser in periodontal pocket therapy. *Br Dent J* 1996: **180**: 57–62.
99. Romanos GE, Everts H, Nentwig GH. Effects of diode and Nd:YAG laser irradiation on titanium discs: a scanning electron microscope examination. *J Periodontol* 2000: **71**: 810–815.
100. Roos-Jansaker AM, Renvert S, Egelberg J. Treatment of peri-implant infections: a literature review. *J Clin Periodontol* 2003: **30**: 467–485.
101. Rovaldi CR, Pievsky A, Sole NA, Friden PM, Rothstein DM, Spacciapoli P. Photoactive porphyrin derivative with broad-spectrum activity against oral pathogens *in vitro*. *Antimicrob Agents Chemother* 2000: **44**: 3364–3367.
102. Sarkar S, Wilson M. Lethal photosensitization of bacteria in subgingival plaque from patients with chronic periodontitis. *J Periodontal Res* 1993: **28**: 204–210.
103. Schafer M, Schmitz C, Horneck G. High sensitivity of *Deinococcus radiodurans* to photodynamically-produced singlet oxygen. *Int J Radiat Biol* 1998: **74**: 249–253.
104. Schuckert KH, Jopp S, Muller U. De novo grown bone on exposed implant surfaces using photodynamic therapy and recombinant human bone morphogenetic protein-2: case report. *Implant Dent* 2006: **15**: 361–365.
105. Schwarz F, Sculean A, Romanos G, Herten M, Horn N, Scherbaum W, Becker J. Influence of different treatment approaches on the removal of early plaque biofilms and the viability of SAOS2 osteoblasts grown on titanium implants. *Clin Oral Investig* 2005: **9**: 111–117.
106. Schwarz F, Sculean A, Rothamel D, Schwenzer K, Georg T, Becker J. Clinical evaluation of an Er:YAG laser for nonsurgical treatment of peri-implantitis: a pilot study. *Clin Oral Implants Res* 2005: **16**: 44–52.
107. Schwarz F, Bieling K, Bonsmann M, Latz T, Becker J. Nonsurgical treatment of moderate and advanced periimplantitis lesions: a controlled clinical study. *Clin Oral Investig* 2006: **10**: 279–288.
108. Schwarz F, Bieling K, Nuesry E, Sculean A, Becker J. Clinical and histological healing pattern of peri-implantitis lesions following non-surgical treatment with an Er:YAG laser. *Lasers Surg Med* 2006: **38**: 663–671.
109. Schwarz F, Jepsen S, Herten M, Sager M, Rothamel D, Becker J. Influence of different treatment approaches on non-submerged and submerged healing of ligature induced peri-implantitis lesions: an experimental study in dogs. *J Clin Periodontol* 2006: **33**: 584–595.
110. Sculean A, Schwarz F, Becker J. Anti-infective therapy with an Er:YAG laser: influence on peri-implant healing. *Expert Rev Med Devices* 2005: **2**: 267–276.
111. Sharman WM, Allen CM, van Lier JE. Photodynamic therapeutics: basic principles and clinical applications. *Drug Discov Today* 1999: **4**: 507–517.
112. Shibli J A, Martins M C, Nociti F H Jr, Garcia V G, Marcantonio E Jr. Treatment of ligature-induced periimplantitis by lethal photosensitization and guided bone regeneration: a preliminary histologic study in dogs. *J Periodontol* 2003: **74**: 338–345.

113. Shibli JA, Martins MC, Theodoro LH, Lotufo RF, Garcia VG, Marcantonio EJ. Lethal photosensitization in microbiological treatment of ligature-induced peri-implantitis: a preliminary study in dogs. *J Oral Sci* 2003: **45**: 17–23.
114. Shibli JA, Martins MC, Ribeiro FS, Garcia VG, Nociti FH Jr, Marcantonio E Jr. Lethal photosensitization and guided bone regeneration in treatment of peri-implantitis: an experimental study in dogs. *Clin Oral Implants Res* 2006: **17**: 273–281.
115. 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.
116. Slots J. The search for effective, safe and affordable periodontal therapy. *Periodontol 2000* 2002: **28**: 9–11.
117. Smetana Z, Malik Z, Orenstein A, Mendelson E, Ben-Hur E. Treatment of viral infections with 5-aminolevulinic acid and light. *Lasers Surg Med* 1997: **21**: 351–358.
118. Smetana Z, Ben-Hur E, Mendelson E, Salzberg S, Wagner P, Malik Z. Herpes simplex virus proteins are damaged following photodynamic inactivation with phthalocyanines. *J Photochem Photobiol B* 1998: **44**: 77–83.
119. Socransky SS, Haffajee AD. Dental biofilms: difficult therapeutic targets. *Periodontol 2000* 2002: **28**: 12–55.
120. 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.
121. Soukos NS, Ximenez-Fyvie LA, Hamblin MR, Socransky SS, Hasan T. Targeted antimicrobial photochemotherapy. *Antimicrob Agents Chemother* 1998: **42**: 2595–2601.
122. Soukos NS, Mulholland SE, Socransky SS, Doukas AG. Photodestruction of human dental plaque bacteria: enhancement of the photodynamic effect by photomechanical waves in an oral biofilm model. *Lasers Surg Med* 2003: **33**: 161–168.
123. 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.
124. Takasaki AA, Aoki A, Mizutani K, Kikuchi S, Oda S, Ishikawa I. Er:YAG laser therapy for peri-implant infection: a histological study. *Lasers Med Sci* 2007: **22**: 143–157.
125. Teichert MC, Jones JW, Usacheva MN, Biel MA. Treatment of oral candidiasis with methylene blue-mediated photodynamic therapy in an immunodeficient murine model. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 2002: **93**: 155–160.
126. Teles RP, Haffajee AD, Socransky SS. Microbiological goals of periodontal therapy. *Periodontol 2000* 2006: **42**: 180–218.
127. Thiha K, Takeuchi Y, Umeda M, Huang Y, Ohnishi M, Ishikawa I. Identification of periodontopathic bacteria in gingival tissue of Japanese periodontitis patients. *Oral Microbiol Immunol* 2007: **22**: 201–207.
128. Umeda M, Takeuchi Y, Noguchi K, Huang Y, Koshy G, Ishikawa I. Effects of nonsurgical periodontal therapy on the microbiota. *Periodontol 2000* 2004: **36**: 98–120.
129. 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* 2001: **29**: 165–173.
130. Usacheva MN, Teichert MC, Biel MA. The interaction of lipopolysaccharides with phenothiazine dyes. *Lasers Surg Med* 2003: **33**: 311–319.
131. Usacheva MN, Teichert MC, Sievert CE, Biel MA. Effect of Ca+ on the photobactericidal efficacy of methylene blue and toluidine blue against gram-negative bacteria and the dye affinity for lipopolysaccharides. *Lasers Surg Med* 2006: **38**: 946–954.
132. van der Meij EH, Schepman KP, van der Waal I. The possible premalignant character of oral lichen planus and oral lichenoid lesions: a prospective study. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 2003: **96**: 164–171.
133. von Tappeiner H, Jodlbauer A. Uber die Wirkung der photodynamischen (fluorescierenden) Stoffe auf Protozoen und Enzyme. *Dtsch Arch Klin Med* 1904: **39**: 427–487.
134. Wainwright M. Photodynamic antimicrobial chemotherapy (PACT). *J Antimicrob Chemother* 1998: **42**: 13–28.
135. Walker C. The acquisition of antibiotic resistance in the periodontal microflora. *Periodontol 2000* 1996: **10**: 79–80.
136. Walker C, Karpinia K. Rationale for use of antibiotics in periodontics. *J Periodontol* 2002: **73**: 1188–1196.
137. Wigdor H, Abt E, Ashrafi S, Walsh JT Jr. The effect of lasers on dental hard tissues. *J Am Dent Assoc* 1993: **124**: 65–70.
138. Williams J, Pearson G, Colles M, Wilson M. The photoactivated antibacterial action of toluidine blue O in a collagen matrix and in carious dentine. *Caries Res* 2004: **38**: 530–536.
139. 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.
140. Wilson M, Mia N. Sensitisation of *Candida albicans* to killing by low-power laser light. *J Oral Pathol Med* 1993: **22**: 354–357.
141. Wilson M, Burns T, Pratten J, Pearson GJ. Bacteria in supragingival plaque samples can be killed by low-power laser light in the presence of a photosensitizer. *J Appl Bacteriol* 1995: **78**: 569–574.
142. 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.
143. Yilmaz S, Kuru B, Kuru L, Noyan U, Argun D, Kadir T. Effect of gallium arsenide diode laser on human periodontal disease: a microbiological and clinical study. *Lasers Surg Med* 2002: **30**: 60–66.
144. Yu CH, Chen HM, Hung HY, Cheng SJ, Tsai T, Chiang CP. Photodynamic therapy outcome for oral verrucous hyperplasia depends on the clinical appearance, size, color, epithelial dysplasia, and surface keratin thickness of the lesion. *Oral Oncol* 2008: **44**: 595–600.
145. Zanin IC, Goncalves RB, Junior AB, Hope CK, Pratten J. Susceptibility of *Streptococcus mutans* biofilms to photodynamic therapy: an *in vitro* study. *J Antimicrob Chemother* 2005: **56**: 324–330.

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22	**AUTHOR: 'microbial reductions' has been rewritten. Please check / approve the changes.**	
23	**AUTHOR: The text 'Sigusch et al. (115) showed that chlorin-e6 plus diode laser also achieved reductions in *P. gingivalis*** in dogs, but failed to reduce F. nucleatum.' has been rewritten. Please check / approve the changes.	
24	**AUTHOR: The text 'Following microbial reductions' has been rewritten. Please check / approve the changes.**	
25	**AUTHOR: The meaning of the text 'exhibited positive results' is unclear. Do you mean something like 'resulted in improvement of the periodontal symptoms' ?**	

26	AUTHOR: 'antimicrobial photodynamic therapy (chlorin-e6 and BLC1010 + diode laser) was distinctly advantageous' – please state what this was being compared with or rephrase the statement.	
27	AUTHOR: 'with' has been changed to 'and resulted in'. Please check / approve the changes.	
28	AUTHOR: The text 'treatment with methylene blue, low-level laser therapy, and methylene blue followed by low-level laser therapy (photodynamic therapy).' has been rewritten. Please check / approve the changes.	
29	AUTHOR: 'alone' has been inserted. Please check / approve the change.	
30	AUTHOR: The text 'which might be partly attributed to the additional photo-bio-modulation effect by low-level laser irradiation of photodynamic therapy' has been rewritten. Please check / approve the changes.	
31	AUTHOR: The meaning of the phrase 'and the nonestablishment of the most effective procedure of antimicrobial photodynamic therapy' is unclear. Do you mean something like 'and fact that the most effective procedure of antimicrobial photodynamic therapy has not been established' ? Please rephrase the original statement for clarity.	
32	AUTHOR: The text 'The authors reported that the adjunctive use of antimicrobial photodynamic therapy with scaling and root planing significantly reduced bleeding on probing one to three months following treatment than the other groups.' has been rewritten. Please check / approve the changes.	
33	AUTHOR: 'in dental implants' has been rewritten as 'in placement of dental implants'. Please check / approve the change.	
34	AUTHOR: The text 'to obtain ideal healing' has been rewritten. Please check / approve the changes.	
35	AUTHOR: 'of' has been inserted	
36	AUTHOR: The meaning of the text 'and the generally insufficient bacterial irradiation as well as poor re-osseointegration following its adjunctive application during nonsurgical and surgical therapy of peri-implantitis' is unclear. Please rephrase.	
37	AUTHOR: 'effects' has been changed to 'efficacy'. Please check / approve the change.	
38	AUTHOR: The text 'Moreover, in some lasers surface alterations, such as melting and carbonization, have been observed on the treated titanium surface following their application' has been rewritten. Please check / approve the changes.	
39	AUTHOR: 'species of' has been inserted. Please check / approve the change.	
40	AUTHOR: The text 'which allows its easy removal after treatment without causing any aesthetic compromise' has been rewritten. Please check / approve the changes.	

41	AUTHOR: The text 'conventional mechanical debridement associated with guided bone regeneration' has been rewritten. Please check / approve the changes.	
42	AUTHOR: The meaning of 'before, after application of toluidine blue O alone' is unclear. Do you mean 'before and after the application of toluidine blue O alone' of 'before the application of toluidine blue O alone' ? Please rephase the original statement for clarity.	
43	AUTHOR: 'alone' has been inserted. Please check / approve the change.	
44	AUTHOR: 'the prevalence of' has been inserted. Please check / approve.	
45	AUTHOR: 'long-time irradiation' has been rewritten. Please check / approve the changes.	
46	AUTHOR: The text ' Thus, in order to use lasers safely within the clinic,' has been rewritten. Please check / approve the changes.	
47	AUTHOR: The text ' Toluidine blue O and methylene blue have been employed as the major photosensitizers' has been rewritten. Please check / approve the changes.	
48	AUTHOR: 'to support this idea' has been inserted. Please check / approve.	
49	AUTHOR: 'Monotherapy with antimicrobial photodynamic therapy' has been rewritten. Please check / approve the change.	
50	AUTHOR: 'without' has been changed to 'when there is no'. Please check / approve the change.	
51	AUTHOR: The text ' attain bacterial eradication from the oral cavity at a much higher level.' has been rewritten. Please check / approve the changes.	
52	AUTHOR: Please provide the volume number, page range for reference [10].	
53	AUTHOR: Please provide the volume number, page range for reference [26].	
54	AUTHOR: Please provide the volume number, page range for reference [33].	
55	AUTHOR: Please provide the volume number, page range for reference [53].	
56	AUTHOR: Please provide the volume number, page range for reference [66].	
57	AUTHOR: 'goes to' has been changed to 'is converted to'. Please check / approve the change.	
58	AUTHOR: Please check that the definition given of 'CW' in the Table 1 footnote is correct.	
59	AUTHOR: 'H+G5ematoporphyrin ester' has been changed to 'hematoporphyrin ester' in the body of Table 2. Please check / approve the change.Please also provide the definition of the abbreviation 'GaAs' listed in the footnote of Table 2.	

60	**AUTHOR: Please check the entries in Table 5.**	

MARKED PROOF

Please correct and return this set

Please use the proof correction marks shown below for all alterations and corrections. If you wish to return your proof by fax you should ensure that all amendments are written clearly in dark ink and are made well within the page margins.

Instruction to printer	*Textual mark*	*Marginal mark*
Leave unchanged	··· under matter to remain	New matter followed by ⌐ or ⌐(x2)
Insert in text the matter indicated in the margin	⌐	
Delete	/ through single character, rule or underline or ⊢⊣ through all characters to be deleted	⌐/ or ⌐/(x2)
Substitute character or substitute part of one or more word(s)	/ through letter or ⊢⊣ through characters	new character / or new characters /
Change to italics	— under matter to be changed	⌐
Change to capitals	≡ under matter to be changed	≡
Change to small capitals	= under matter to be changed	=
Change to bold type	~ under matter to be changed	~
Change to bold italic	≈ under matter to be changed	≈
Change to lower case	Encircle matter to be changed	≠
Change italic to upright type	(As above)	⌐
Change bold to non-bold type	(As above)	⌐
Insert 'superior' character	/ through character or ⌐ where required	Y or Y under character e.g. Y^{3} or Y^{3}
Insert 'inferior' character	(As above)	⌐ over character e.g. Y_{2}
Insert full stop	(As above)	⊙
Insert comma	(As above)	,
Insert single quotation marks	(As above)	Y or Y and/or Y or Y
Insert double quotation marks	(As above)	Y or Y and/or Y or Y
Insert hyphen	(As above)	⊢
Start new paragraph	⌐	⌐
No new paragraph	⌐	⌐
Transpose	⌐	⌐
Close up	linking ⁀ characters	⁀
Insert or substitute space between characters or words	/ through character or ⌐ where required	Y
Reduce space between characters or words	| between characters or words affected	⊤