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Photodynamic therapy in the control of oral biofilms

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Microbial biofilms in the oral cavity are involved in the etiology of various oral conditions, including caries, periodontal and endodontic diseases, oral malodor, denture stomatitis, candidiasis and dental implant failures. It is generally recognized that the growth of bacteria in biofilms imparts a substantial decrease in susceptibility to antimicrobial agents compared with cultures grown in suspension (39). It is therefore not surprising that bacteria growing in dental plaque, a naturally occurring biofilm (127), display increased resistance to antimicrobial agents (4, 67). Current treatment techniques involve either periodic mechanical disruption of oral microbial biofilms or maintaining therapeutic concentrations of antimicrobials in the oral cavity, both of which are fraught with limitations. The development of alternative antibacterial therapeutic strategies therefore becomes important in the evolution of methods to control microbial growth in the oral cavity.

The use of photodynamic therapy for inactivating microorganisms was first demonstrated more than 100 years ago, when Oscar Raab (164) reported the lethal effect of acridine hydrochloride and visible light on *Paramecia caudatum*. Photodynamic therapy for human infections is based on the concept that an agent (a photosensitizer) which absorbs light can be preferentially taken up by bacteria and subsequently activated by light of the appropriate wavelength (Fig. 1) in the presence of oxygen to generate singlet oxygen and free radicals that are cytotoxic to microorganisms (Fig. 2). Because of the primitive molecular nature of singlet oxygen, it is unlikely that microorganisms would develop resistance to the cytotoxic action. Photodynamic therapy has emerged as an alternative to antimicrobial regimes and mechanical means in eliminating dental plaque species as a result of the pioneering work of Professor Michael Wilson and colleagues (223) at the Eastman Dental Institute, University College London, UK.

In this review, we propose to provide an overview of photodynamic therapy with emphasis on its current status as an antimicrobial therapy to control oral bacteria, and review the progress that has been made in the last 15 years concerning the applications of photodynamic therapy for targeting biofilm-associated oral infections. Problems and challenges that have arisen will be identified and discussed. Finally, new frontiers of antimicrobial photodynamic therapy research will be introduced, including targeting strategies that may open new opportunities for the maintenance of bacterial homeostasis in dental plaque, thereby providing the opportunity for more effective disease prevention and control.

Photodynamic therapy: an overview

Mechanism of photodynamic therapy action

The involvement of light and oxygen in the photodynamic process was demonstrated at the start of the last century by von Tappeiner (213), who coined the term ‘photodynamic’. Following absorption of a photon of light, a molecule of the photosensitizer in its ground singlet state (S) is excited to the singlet state (S*) and receives the energy of the photon (Fig. 3). The lifetime of the S* state is in the nanosecond range, which is too short to allow significant interactions with the surrounding molecules (50, 102). The S* state molecule may decay back to the ground state by emitting a photon as light energy (fluorescence) or by internal conversion with energy lost as heat. Alternatively, the molecule may convert into an excited triplet state (T) molecule via intersystem crossing that involves a change in the spin of an electron (147). The lifetime of the T state is in the

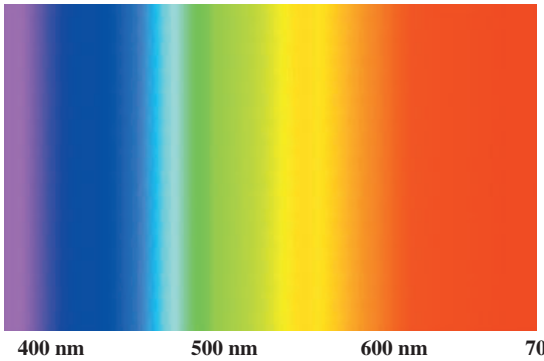


Fig. 1. Visible light, which covers the range of 400–700 nm of all electromagnetic radiation, is most relevant to photodynamic therapy. However, in practice, the range of light used in photodynamic therapy is generally >600 nm. This is because endogenous molecules, such as hemoglobin, absorb light strongly at wavelengths of <600 nm and therefore capture most of the incoming photons.

microsecond to the millisecond range. Molecules in the T state can emit light (phosphorescence) by returning to the ground state or can react further by one or both of two pathways (known as the Type I and Type II photoprocesses), both of which require oxygen (147). The Type I reaction involves electron-transfer reactions from the photosensitizer triplet state with the participation of a substrate to produce radical ions that can react with oxygen to produce cytotoxic species, such as superoxide, hydroxyl and lipid-derived radicals (5). The Type II reaction involves energy transfer from the photosensitizer triplet state to ground state molecular oxygen (triplet) to produce excited state singlet oxygen, which can oxidize many biological molecules, such as proteins, nucleic acids and lipids, and lead to cytotoxicity (167). Singlet oxygen, probably the major damaging species in photodynamic therapy (102), has a diffusion distance of approximately 100 nm (137) and a half-life of <0.04 μs (138). There are several factors influencing photodamage, including the type, dose, incubation time and localization of the photosensitizer, the availability of oxygen, the wavelength of light (nm), the light power density (mW/cm^2) and the light energy fluence (J/cm^2). An important characteristic of photodynamic therapy is its inherent dual selectivity; first by achieving an increased concentration of the photosensitizer by specific binding to target tissue and, second, by constraining the irradiation to a specified volume. In antibacterial photodynamic therapy, photodestruction is mainly caused by damage to the cytoplasmic membrane and DNA (8, 171, 176).

Photosensitizers

Most of the photosensitizers (Fig. 4) under investigation for cancer treatment are based on the tetrapyrrole nucleus, such as porphyrins, chlorins, bacteriochlorins and phthalocyanines (214). This tetrapyrrole ring structure is named porphin and derivatives of porphins are named porphyrins (Fig. 4). Porphyrins comprise four pyrrole subunits linked together by four methane bridges. Chlorins and bacteriochlorins are porphyrins with one and two reduced double bonds, respectively, whereas phthalocyanines and naphtalocyanines are porphyrins with an extended ring system (Fig. 4). Table 1 provides a list of the photosensitizers that have been approved for clinical use. The first approved photosensitizer was hematoporphyrin derivative (Photofrin®; Axcan Pharma Inc., Mont-Saint-Hilaire, Canada) for the treatment of refractory superficial

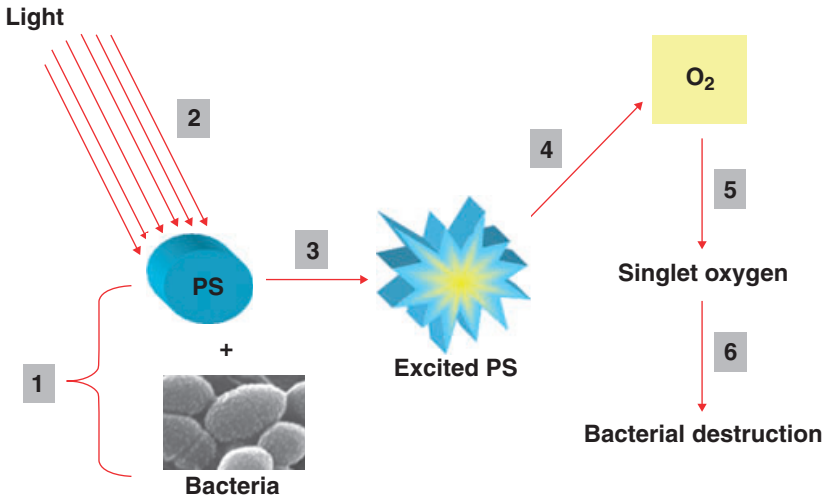


Fig. 2. Mechanism of photodynamic therapy action. A photosensitizer is taken up by microorganisms (1) and following exposure to light of the appropriate wavelength (2) becomes activated to an excited state (3). Then, the photosensitizer transfers energy from light to molecular oxygen (4) to generate singlet oxygen and free radicals (5) that are cytotoxic to cells (6).

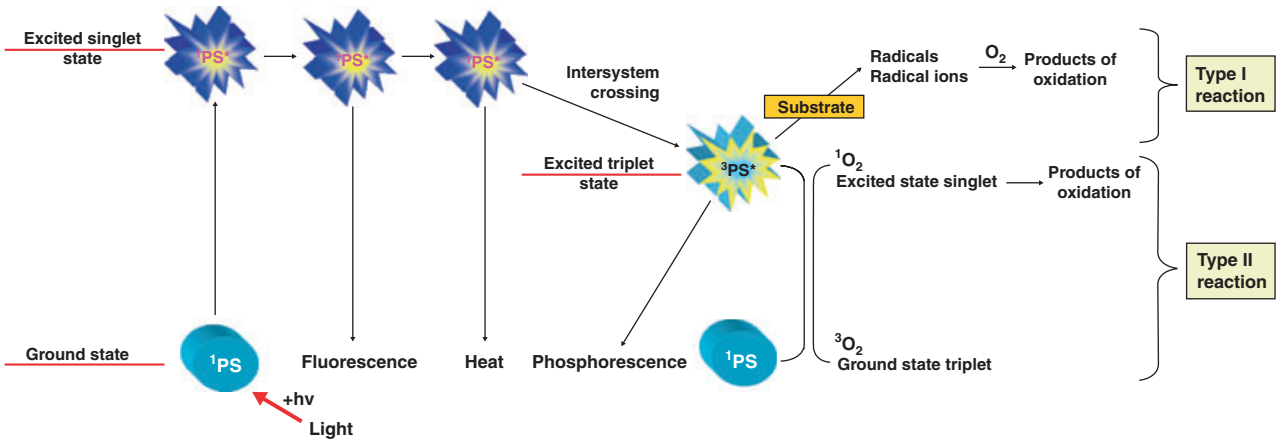


Fig. 3. Type I and Type II reactions in photodynamic therapy. Following exposure to light, the activated photosensitizer (in the excited triplet state) can follow one of two pathways. The Type I pathway involves electron-transfer reactions from the photosensitizer triplet state with the participation of a substrate to produce radical ions that can react with oxygen to produce cytotoxic species. The Type II pathway involves energy transfer from the photosensitizer triplet state to the ground state molecular oxygen (triplet) to produce excited state singlet oxygen, which can oxidize biological molecules. hv, photon energy; PS, photosensitizer.

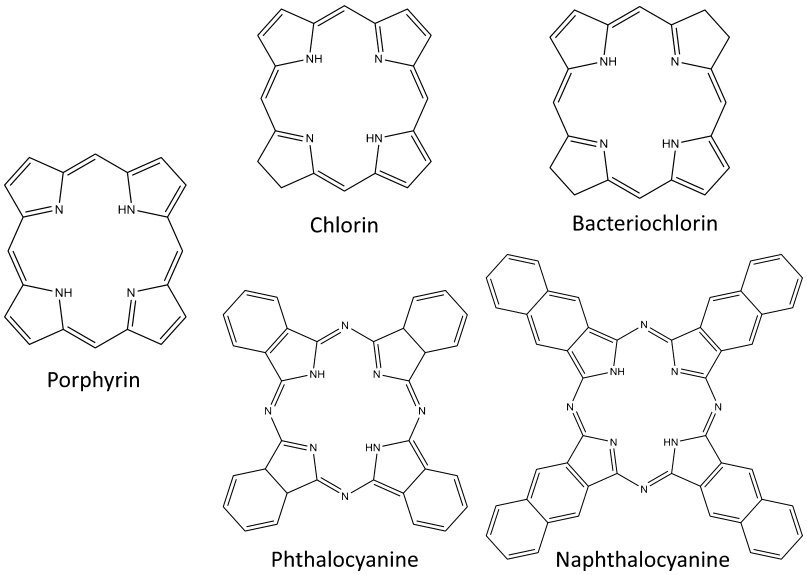
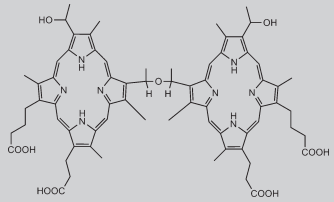
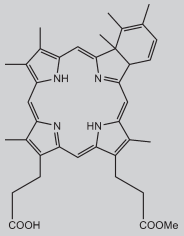
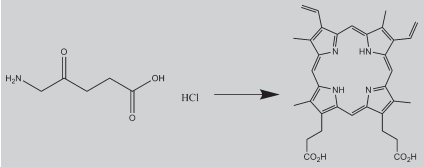
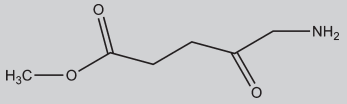
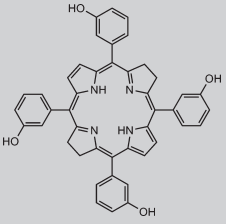


Fig. 4. Basic structure of porphyrin-based photosensitizers.

bladder cancer (207). Disadvantages related to the use of photofrin include prolonged cutaneous phototoxicity (4–6 weeks), its poor chemical characterization and the low absorption in the wavelength region of therapeutic interest. Other photosensitizers are now approved for clinical use, including *meso*-tetra-hydroxyphenyl-chlorin (mTHPC, temoporfin, Foscan®; Biolitec Pharma Ltd., Dublin, Ireland), benzoporphyrin derivative monoacid A (BPD-MA, Visudyne®; QLT Inc., Vancouver, Canada and Novartis Opthalmics, Bulach, Switzerland), 5- or δ-aminolevulinic acid (ALA, Levulan®; DUSA Pharmaceuticals Inc., Wilmington, MA, USA) and the methyl ester of ALA (Metvix®; Photocure ASA, Oslo, Norway) (207). The latter two agents are not photosensitizers but are prodrugs converted by the body to protoporphyrin IX (or the methyl derivative for Metvix) via the heme biosynthetic pathway when applied topically. The advantage here is that the administration of ALA only temporarily overloads the natural synthetic pathway, and therefore photosensitization lasts for no longer than several hours. However, the use of ALA is restricted to superficial premalignant lesions (up to 2 mm) as a result of the limited penetration of ALA and the limited penetration of light at 635 nm that activates protoporphyrin IX.

In antimicrobial photodynamic therapy, a photosensitizer ideally should possess the following properties: a high quantum yield of triplet state to obtain large concentrations of the activated drug; a

Table 1. Approved photosensitizers for use in photodynamic therapy

Chemical name, abbreviation, trade name and chemical structure	Source and description	Use and wavelength
Hematoporphyrin derivative (porfimer sodium) Photofrin® 	Axcan Pharma http://www.axcan.com A proprietary combination of monomers, dimers and oligomers derived from the chemical manipulation of hematoporphyrin	Esophageal, cervical, gastric, lung superficial bladder and endobronchial malignancies Approved for systemic use in more than 100 countries 632 nm
Benzoporphyrin-derivative monoacid Ring A (BPD-MA, verteporfin) Visudyne® 	QLT Inc. and Novartis Opthalmics http://www.qltinc.com A hydrophobic molecule that is distinguished by the presence of a mono-acid at either position 3 or 4 of the porphyrin ring	Certain forms of choroidal neo-vascularization are caused by wet age-related macular degeneration, pathological myopia, or ocular histoplasmosis Approved for systemic use worldwide 690 nm
5-Aminolevulinic acid (ALA) Levulan® 	DUSA Pharmaceuticals, Inc. http://www.dusapharma.com The hydrochloride salt of ALA is a prodrug, a naturally occurring amino acid that is converted enzymatically to protoporphyrin IX (PpIX)	Actinic keratosis of face or scalp Approved for topical use in the USA 632 nm
Methylaminolevulinate (MAL) Metvix® 	Galderma http://www.galderma.com MAL is the methylated ester of ALA	Actinic keratosis, basal cell carcinoma (approved for topical use in the European Union, Australia, New Zealand, Brazil) Actinic keratosis (approved for topical use in the USA) 632 nm
Meta-tetra hydroxyphenyl chlorine (m-THPC, temoporfin) Foscan® 	Biolitec AG http://www.biolitecpharma.com Contains a hydrophobic chlorin core and hydroxyphenyl groups at the meso position for increased solubility	Palliative treatment of patients with advanced head and neck cancer Approved for systemic use in the European Union, Norway and Iceland) 652 nm

high singlet oxygen quantum yield; high binding affinity for microorganisms; a broad spectrum of action; low binding affinity for mammalian cells to avoid the risk of photodestruction of host tissues; a low propensity for selecting resistant bacterial strains; a minimal risk of promoting mutagenic processes; and low chemical toxicity (94). Gram-positive bacteria are generally susceptible to photoinactivation (9, 11, 124), whereas gram-negative bacteria are often reported to be resistant to photodynamic action (11, 123), unless the permeability of their outer membrane is modified (10, 145).

Antimicrobial photosensitizers such as porphyrins, phthalocyanines and phenothiazines (e.g. toluidine blue O and methylene blue), which bear a positive charge, can directly target both gram-negative and gram-positive bacteria (131, 136, 226). The positive charge seems to promote the binding of the photosensitizer to the outer bacterial membrane, inducing localized damage, which favors its penetration (132). Toluidine blue O and methylene blue (Fig. 5) are commonly used for oral antimicrobial photodynamic therapy. Toluidine blue O is a vital dye that has been used for staining mucosal abnormalities of the uterine cervix and oral cavity and for demarcating the extent of lesions before excision (119). In addition, it has been shown to be a potent photosensitizer for killing oral bacteria (226). Methylene blue has been used as a photosensitizing agent since the 1920s (215). It has been used to detect mucosal premalignant lesions (150) and as a marker dye in surgery (37). The hydrophilicity of methylene blue (216), along with its low molecular weight and positive charge, allows passage across the porin-protein channels in the outer membrane of gram-negative bacteria (210). Methylene blue, the intravenous administration of which is approved by the US Food and Drug Administration for methemoglobinemia, interacts predominantly with the anionic macromolecule lipopolysaccharide, resulting in the generation of methylene blue dimers (210), which participate in the photosensitization process (6, 210). Recently, the activation of photosensitizers has been achieved by diode lasers emitting light of a specific wavelength. These devices are portable and their cost is much lower compared with that of argon lasers, gallium-aluminum-arsenide diode lasers and helium-neon lasers, which have been mostly employed in photodynamic therapy.

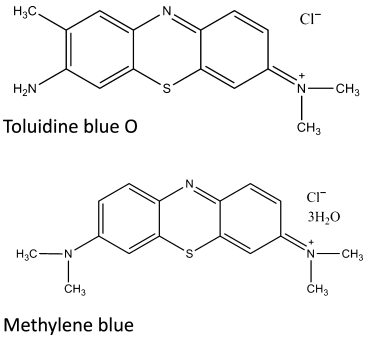


Fig. 5. Chemical structures of the phenothiazine photosensitizers toluidine blue O, $C_{15}H_{16}N_3SCl$ (also known as tolonium chloride, basic blue 17, blutene chloride and methylene blue T50 or T extra) and methylene blue, $C_{16}H_{18}ClN_3S$ (also known as methylthionine chloride and 3,7-bis(Dimethylamino)-phenazothionium chloride).

Current photodynamic therapy status

Photodynamic therapy has found its greatest success in the treatment of cancer (207), age-related macular degeneration (20), actinic keratosis (208) and Barrett's esophagus (154) (Table 1). The application of photodynamic therapy for targeting pathogenic microbes in wound infections has been explored in animal models (82, 83, 108, 152, 237). Photodynamic therapy with topical application of ALA is used off-label for the treatment of acne vulgaris (76, 90) and has been employed for clinical use as an antifungal agent (30).

In the dental field, photodynamic therapy is approved for the palliative treatment of patients with advanced head and neck cancer in the European Union, Norway and Iceland. Recently, in Canada, the product called Periowave (http://www.periowave.com) was commercialized by Ondine Biopharma Corporation (http://www.ondinebiopharma.com) for the treatment of periodontitis. The Periowave product consists of a laser system with a custom-designed handpiece and patient treatment kits of methylene blue. A kit that includes phenothiazine chloride for clinical photodynamic therapy is now available in Austria, Germany, Switzerland and the UK (Helbo®; Photodynamic Systems GmbH & Co. KG, Grieskirchen, Austria). Similar kits that include toluidine blue O are also available from other companies, including Denfotex Ltd., Dexcel Pharma Technologies Ltd., SciCan Medtech AG and Cumdente GmbH.

Phototargeting oral biofilms

Dental caries

Dental caries results from an ecological imbalance in the physiological equilibrium between tooth minerals and oral microbial biofilms, mainly supragingival plaque (178). Biofilm bacteria, such as mutans streptococci (*Streptococcus mutans* and *Streptococcus sobrinus*) and *Lactobacillus* species, secrete organic acids as a by-product of the metabolism of fermentable carbohydrates. This process leads to the demineralization of tooth hard-tissue cavitation in its advanced stages (58). Management of early carious lesions includes preventive approaches, such as dental plaque removal, through dental home care (toothbrushing, antimicrobials), professional place-

ment of sealants and topical fluoride applications. Treatment of cavitated lesions involves the surgical removal of the infected tooth structure followed by tooth restoration. Photodynamic therapy could be used as dental caries preventive by targeting dental-fermentative plaque microorganisms and as a minimally invasive technique to eliminate bacteria within carious lesions (224). This technique could offer the following benefits: rapid noninvasive topical *in vivo* application of the drug to the carious lesion; rapid bacterial killing after a short exposure to light; unlikely development of resistance considering the widespread generic toxicity of reactive oxygen species; and confined killing by restricting the field of irradiation and the inherently short diffusion radius of reactive oxygen species.

Several laboratory studies have demonstrated (using toluidine blue O) the susceptibility of cariogenic bacteria, either in the planktonic phase (12, 23, 24, 220) or in the biofilm phase (75, 233, 234), to photodynamic therapy. Toluidine blue O and light effectively reduced the number of microorganisms in supragingival dental plaque samples obtained from human subjects (226). Toluidine blue O-induced photodynamic therapy was able to achieve a 10-fold reduction of *S. mutans* when the organism was embedded in a collagen matrix mimicking carious dentin or present in decayed teeth (25, 221). Rose Bengal, a fluorescent dye that is used to study liver function, has been employed to target *S. mutans* species in suspension (158), and disulfonated aluminium phthalocyanine ($AlPcS_2$) has been shown to be effective against suspensions (25) and biofilms of cariogenic bacteria (225) as well as against human supragingival dental plaque microbes in the planktonic phase (226). The synergistic effect of erythrosine, a dental plaque-disclosing agent currently in clinical use, and photodynamic therapy, induced bacterial cell killing of >1.5 $\log_{10}$ in *S. mutans* biofilms *in vitro* (133, 229). Recently, the combined application of photodynamic therapy and casein phosphopeptide-amorphous calcium phosphate, a compound with established remineralization capabilities (168), proved to be a successful treatment approach in removing the cariogenic bacteria and arresting root surface caries *in vivo* (212). In addition, it has been demonstrated that the combination of toluidine blue O and red light with energy fluencies at 47 and 94 J / cm^2 resulted in a significant reduction of cariogenic species present in dentine caries produced *in situ* (117).

Photodynamic therapy carries promise for targeting cariogenic bacteria. The data obtained from *in vitro* studies are encouraging; however, a lack of reliable clinical trial evidence has not allowed photodynamic therapy to be confirmed as an effective method for the prevention, control and treatment of caries. Not all laboratory photodynamic therapy studies have been effective in reducing caries organisms. For example, methylene blue-induced photodynamic therapy was not able to reduce significantly the load of microorganisms in an *in vitro* multispecies biofilm model comprising cariogenic bacteria (142). More clinical and laboratory studies are needed to explore the anticariogenic potential of photodynamic therapy and to establish the optimum treatment parameters.

Periodontal diseases

Biofilms that colonize tooth surfaces and epithelial cells lining the periodontal pocket / gingival sulcus (subgingival dental plaques) are among the most complex biofilms that exist in nature. These biofilms include a subset of selected species from more than 700 bacterial species or phylotypes (106, 107, 174) that can lead to periodontal diseases (gingivitis or periodontitis). Mechanical removal of the periodontal biofilms is currently the most frequently used method of periodontal disease treatment. Antimicrobial agents are also used, but biofilm species exhibit several resistance mechanisms (4, 46, 67) and maintaining therapeutic concentrations of antimicrobials in the oral cavity can be difficult (224).

Photodynamic therapy has been suggested as an alternative to chemical antimicrobial agents to eliminate subgingival species and treat periodontitis (223). The application of methylene blue-mediated photodynamic therapy in clinical studies using either the Periowave™ Treatment kit or the Helbo® Blue treatment kit is as follows: methylene blue is applied directly in the dental pockets for 60 s followed by exposure to red light via a fiberoptic probe for 60 s per pocket or per tooth (10 s per site, six sites in total). In the majority of these studies, photodynamic therapy as an adjunct to scaling and root planing did not show any beneficial effects over scaling and root planing alone. It is possible that short exposures to light may be responsible for the lack of clinical benefits. Data obtained from these studies are presented in Table 2 and will be discussed below.

Several studies have shown that periodontal bacteria are susceptible to photodynamic therapy in planktonic cultures (14, 15, 31, 128, 195, 227), plaque scrapings (175, 226) and biofilms (47, 230) using methylene blue (31, 47, 227), toluidine blue O (14, 15,

47, 128, 175, 226, 227), phthalocyanine (47, 226, 230), hematoporphyrin HCl (47), hematoporphyrin ester (47) and a conjugate between poly-L-lysine and the photosensitizer chlorin e6 (195). Other studies, however, have demonstrated incomplete destruction of oral pathogens in plaque scrapings (163, 191), monospecies biofilms (191, 192) and multispecies biofilms (142, 149, 151) using methylene blue (142, 149, 192), toluidine blue O (151, 163) and poly-L-lysine/photosensitizer chlorin e6 (191). The susceptibility of bacteria derived from human natural dental plaque to methylene blue-mediated photodynamic therapy *in vitro* under planktonic or biofilm conditions was compared (64). In this study, the microcosm biofilms originated directly from the whole-mixed natural dental plaque and were developed on agar surfaces in 96-well plates. Suspensions of plaque microorganisms from five subjects were sensitized with methylene blue (25 μg/ml) for 5 min and then exposed to red light. Biofilms were also exposed to methylene blue (25 or 50 μg/ml) and the same light conditions as their planktonic counterparts. Photodynamic therapy produced approximately 63% killing of bacteria in the planktonic phase, whereas in biofilms derived from the same plaque samples the effect of light was reduced (31% killing). The reduced susceptibility of bacteria to photodynamic therapy in the planktonic phase may be related to the distinct and protected phenotypes expressed by them once they attach to the tooth (40), which are still carried by dental plaque bacteria in suspension. It has also been shown that phenothiazine-based photosensitizers, including methylene blue and toluidine blue O, are substrates of multidrug resistance pumps in bacteria (204). The reduced susceptibility of biofilms to photodynamic therapy may be related to the inactivation of methylene blue (62), the existence of biofilm bacteria in a slow-growing or starved state (22) and to certain phenotypes expressed by biofilm species when they attach to the agar surface (218). The reduced susceptibility of biofilms to photodynamic therapy may also be attributed to the reduced penetration of methylene blue, an explanation that has been introduced previously (200). It has been suggested, in studies of model systems, that water channels can carry solutes into or out of the depths of a biofilm, but they do not guarantee access to the interior of the cell clusters (199) whose diameter may range from 20 to 600 μm (166).

Biophysical means, such as ultrasonic irradiation (162) and electric fields (36), known as the 'bioacoustic' effect and the 'bioelectric' effect, respectively, have been employed to enhance the efficacy of various agents in killing biofilm microorganisms. These methodologies, however, require an application time of up to 48 h in order to achieve significant bacterial killing (26, 27), which would preclude their clinical use. Photomechanical waves are unipolar compression waves generated by lasers (51) and are one of the latest technology platforms for drug delivery.

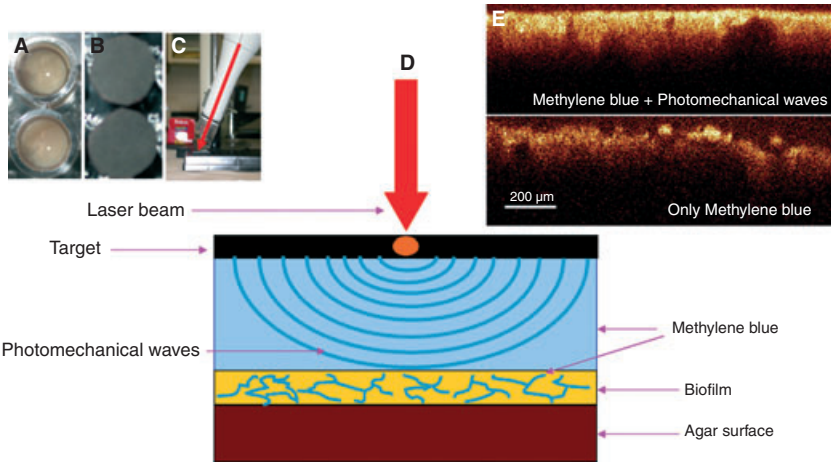


Fig. 6. Saliva-derived microcosm biofilms with a thickness of 200–220 μm were developed on agar (A). Methylene blue was applied onto biofilms and a target (black polystyrene) was placed carefully on the well in contact with the methylene blue surface (B). The laser pulses from a Q-switched Nd:YAG laser were delivered with an articulated arm and completely absorbed by the target (C). Photomechanical waves were generated by ablation of the target material, propagated through the dye solution (which acts as the acoustic coupling medium) and impinged onto the biofilm (D). Confocal fluorescence imaging (*X–Z*) (E) demonstrated a stronger fluorescent signal obtained from biofilms treated with methylene blue and photomechanical waves (above) compared with those treated with methylene blue only (below). The application of photomechanical waves also enhanced the penetration depth of methylene blue (149).

Table 2. Clinical photodynamic therapy studies for treatment of periodontitis

Study and goal	Design, photosensitizer and method	Results
Yilmaz et al., 2002 (232) Effects of a single session of MB-mediated PDT and/or mechanical subgingival debridement on the proportions of obligate anaerobes, plaque indices, bleeding on probing and probing pocket depth	A randomized clinical study with a split-mouth design; 10 subjects with chronic periodontitis MB (50 μg/ml) was applied as a mouth rinse for 60 s followed by exposure of each papillary region to light at 685 nm from a 30 mW diode laser for 71 s	No additional microbiological and clinical benefits over conventional mechanical debridement over a period of 32 days
Andersen et al., 2007 (3) Effects of a single session of MB-mediated PDT (Periowave™ Treatment kit) and/or SRP on bleeding on probing, probing pocket depth and clinical attachment level	A randomized clinical study; 33 subjects with chronic periodontitis MB (50 μg/ml) was applied in each site into periodontal pockets for 60 s followed by exposure to light at 670 nm from a 50 mW diode laser for 60 s	SRP combined with PDT led to significant improvements of the investigated parameters over the use of SRP alone over a period of 3 months
de Oliveira et al., 2007 (45) Effects of a single session of MB-mediated PDT (Helbo® Blue treatment kit) or SRP on plaque index, gingival index, bleeding on probing, probing depth, gingival recession and clinical attachment level	A randomized clinical study with a split-mouth design; 10 subjects with aggressive periodontitis Irrigation with MB (10 mg/ml) for 1 min was followed by exposure to light at 660 nm from a diode laser (60 mW/cm^2) for 1 min per tooth (10 s per site, six sites in total)	PDT and SRP showed similar clinical results over a period of 3 months
Braun et al., 2008 (19) Effects of a single session of MB-mediated PDT (Helbo® Blue treatment kit) and/or SRP on relative attachment level, probing depth and gingival recession and sulcus fluid flow rate	A randomized clinical study with a split-mouth design; 20 subjects with chronic periodontitis Irrigation of pockets with MB (10 mg/ml) for 3 min was followed by exposure to light at 660 nm from a 100 mW diode laser for 1 min per tooth (10 s per site, six sites in total)	All clinical parameters were significantly improved by adjunctive PDT 3 months after treatment
Christodoulides et al., 2008 (34) Effects of a single session of MB-mediated PDT (Helbo® Blue treatment kit) and/or SRP on full-mouth plaque score, full-mouth bleeding score, probing depth, gingival recession, clinical attachment and load of 11 periodontal pathogens	A randomized clinical trial (initial treatment); 24 subjects with chronic periodontitis Irrigation of pockets with MB (10 mg/ml) for 3 min was followed by exposure to light at 670 nm from a 75 mW diode laser (the tip was moved circumferentially around each tooth for 1 min)	PDT and SRP resulted in a significantly greater reduction in bleeding scores compared with scaling and root planing alone over a period of 6 months
de Oliveira et al., 2009 (44) Effects of a single session of MB-mediated PDT (Helbo® Blue treatment kit) or SRP on cytokine levels (tumor necrosis factor- RANKL) in the gingival crevicular fluid	A randomized clinical study with a split-mouth design; 10 subjects with aggressive periodontitis Irrigation with MB (10 mg/ml) for 1 min was followed by exposure to light at 660 nm from a diode laser (60 mW/cm^2) for 1 min per tooth (10 s per site, six sites in total)	PDT and SRP demonstrated similar reductions in the tumor necrosis factor-α and RANKL levels at 30 days
Polansky et al., 2009 (161) Effects of a single session of MB-mediated PDT (Helbo® Blue treatment kit) and/or subgingival ultasound on gingival index, bleeding on probing, probing pocket depths, clinical attachment level and load of *Porphyromonas gingivalis*, *Tannerella forsythia* and *Treponema denticola*	A randomized-controlled clinical pilot trial; 58 subjects with chronic periodontitis Irrigation of each site with MB (10 mg/ml) for 3 min was followed by exposure to light at 680 nm from a 75 mW diode laser for 1 min per tooth surface (mesial, distal, lingual, buccal)	No additional clinical or microbiological benefits of PDT over a period of 3 months

Table 2. (Continued)

Study and goal	Design, photosensitizer and method	Results
Chondros et al., 2009 (33) Effects of a single session of MB-mediated PDT (Helbo® Blue treatment kit) and/or SRP on full-mouth plaque score, full-mouth bleeding score, bleeding on probing, probing depth, gingival recession, clinical attachment and load of 11 periodontal pathogens	A randomized clinical trial; 24 maintenance patients with chronic periodontitis Irrigation of pockets with MB (10 mg/ml) for 3 min was followed by exposure to light at 670 nm from a diode laser (75 mW/cm^2) (the tip was moved circumferentially around each tooth for 1 min)	PDT and SRP resulted in a significantly greater reduction in bleeding scores and in a significant increase in the number of *Eikenella corrodens* and *Capnocytophaga* species at 6 months
Lulic et al., 2009 (122) Effects of repeated (fivr times within two weeks) MB-mediated PDT (Helbo® Blue treatment kit) and/or SRP on probing pocket depth, clinical attachment level and bleeding on probing	A randomized-controlled clinical trial with double-blind design; 10 maintenance patients with chronic periodontitis Irrigation of pockets with MB (10 mg/ml) for 3 min was followed by exposure to light at 670 nm from a diode laser (75 mW/cm^2) (the tip was moved circumferentially around each tooth for 1 min)	Repeated PDT as an adjunct to mechanical debridement led to significantly improved outcomes in all clinical parameters at 6 months
Al-Zahrani et al., 2009 (2) Effects of a single session of MB-mediated PDT (Periowave™ Treatment kit) and/or SRP, and SRP + systemic doxycycline on plaque and bleeding scores, probing pocket depth, clinical attachment level and glycosylated hemoglobin level	A randomized clinical study; 45 subjects with type 2 diabetes and moderate to severe chronic periodontitis MB (50 μg/ml) was applied in each site into periodontal pockets for 5–10 s followed by exposure to light at 670 nm for 60 s	No added benefit of PDT on clinical parameters or glycemic control was found over a period of 3 months
Rühling et al., 2009 (172) Effects of a single session of MB-mediated PDT or subgingival ultrasound on plaque index, probing pocket depth, relative attachment level, bleeding on probing and load of six periodontal pathogens	A randomized, controlled, single-blind clinical study; 54 maintenance patients with chronic periodontitis MB (50 μg/ml) was applied in each site into periodontal pockets for 30 s followed by exposure to light at 635 nm from a 100 mW diode laser for 60 s	No additional clinical or microbiological benefits of PDT over a period of 3 months

MB, methylene blue; PDT, photodynamic therapy; SRP, scaling and root planing.

Photomechanical waves have been used to deliver macromolecules (including genes) through the cell plasma membrane (112, 148, 206), the nuclear envelope (118), the skin (52, 113) and the oral monospecies biofilms (191, 192). The increased delivery of photosensitizers in oral monospecies biofilms by photomechanical waves was correlated with an increased level of bacterial killing by red light *in vitro* (191, 192). Recently, we showed that the application of photomechanical waves also enhanced the methylene blue concentration and the penetration depth into multispecies biofilms evolved from human saliva *in vitro* (149) (Fig. 6). Our hypothesis was that photomechanical waves enhance fluid forces at the biofilm–bulk water interface that deform the microcolonies of bacteria and the matrix, so that fluid movement occurs. The synergistic action of photomechanical waves and photodynamic therapy has the potential to contribute to the development of a new system for the topical, rapid and noninvasive treatment of periodontitis. In a clinical setting, both technologies would be applied in the dental pocket using fiberoptics. However, the optimal parameters of photomechanical waves, such as rise time, peak pressure and number of pulses, for complete eradication of microorganisms in oral microcosm biofilms, remain to be determined. It has been shown that photomechanical wave-induced delivery in different biological systems was affected by these parameters (114, 141).

In vivo studies with experimentally induced periodontits in rats have shown suppression of peri-

odontal pathogens and a reduction of periodontitis following photodynamic therapy with toluidine blue O (100, 163). However, de Almeida et al. (42, 43) found that photodynamic therapy had a short-term effect on the reduction of periodontal tissue destruction in rats. The same authors also found significant reductions of periodontal bone loss in diabetic (41) and immunosuppressed (59) rats using toluidine blue O. Several clinical studies have been carried out to investigate the effects of adjunctive photodynamic therapy in human periodontitis (Table 2). In all of these studies, methylene blue was the photosensitizer. Two of these studies reported significant clinical improvement (reduced probing pocket depth and bleeding on probing, increased clinical attachment level) when photodynamic therapy was used with scaling and root planing (3, 19). Repeated photodynamic therapy (five times within 2 weeks) as an adjunct to mechanical debridement also led to significantly improved clinical effects (122). Other studies have not reported significant clinical benefits (2, 33, 34, 161, 232). Recently, it was reported that either photodynamic therapy or scaling and root planing alone had similar effects on clinical parameters (45, 172) as well as on tumor necrosis factor-alpha and receptor activator of nuclear factor-kappaB ligand (RANKL) in the gingival crevicular fluid of patients with aggressive periodontitis (44). The safety of topical oral antimicrobial photodynamic therapy using toluidine blue O has been demonstrated in several studies (99, 121, 194). In addition, the clinical use of methylene blue for the photodynamic therapy of bladder (222) and esophageal (153) cancers, along with its use in phototargeting *Helicobacter pylori* in the rat gastric mucosa (135), suggest that the local use of methylene blue is safe. Further studies are needed to determine effective parameters for maximum clinical benefit that leave periodontal tissues intact. Clinical studies supporting their efficacy and safety, however, are still in short supply.

Peri-implantitis

Plaque-induced peri-implantitis is an inflammatory condition that affects soft and hard tissues surrounding an osseointegrated dental implant and may lead to its failure (115, 120). The incidence of peri-implantitis in patients with chronic periodontitis is up to five times greater than in patients who are free of this disease (96). In addition, greater proportions of periodontal pathogens have been found in infected and failing implants compared with nonfailing implants (139). The management of peri-implantitis includes the mechanical removal of biofilm from the implants, the local application of antiseptics and antibiotics to kill bacteria in the surrounding peri-implant tissues, and regenerative surgery to help re-establish the bone–implant interface (109).

A limited number of animal (86, 179–181) and clinical (49, 80) studies have reported the effects of antimicrobial photodynamic therapy as an adjunct to

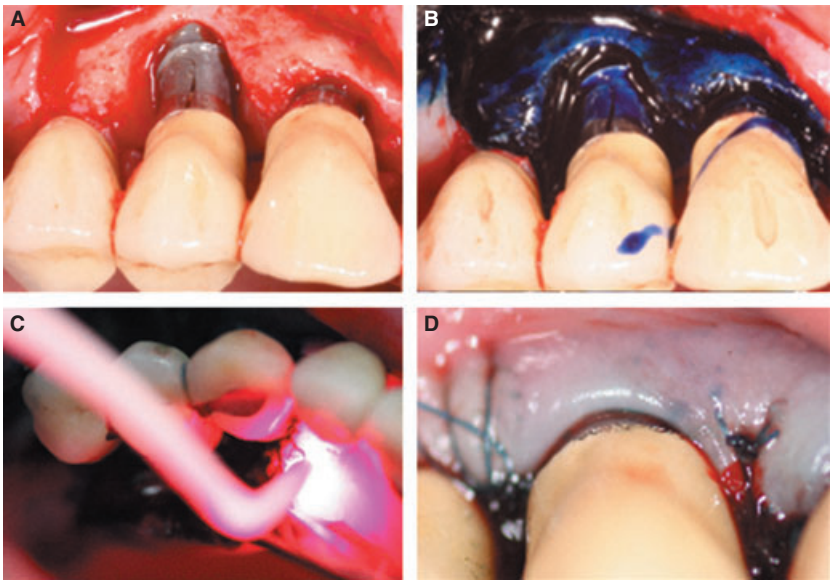


Fig. 7. Clinical application of photodynamic therapy as an adjunct to the surgical treatment of peri-implantitis. (A) Appearance of the intrabony defect around the implant, as observed during access flap surgery and after the removal of granulation tissue. (B) Application of methylene blue. (C) Activation of the dye with the diode laser light (wavelength: 670 nm). (D) Flap closure with vertical mattress sutures.

the treatment of peri-implantitis using toluidine blue O (49, 80, 179–181) and Azulene (86). In two studies, photodynamic therapy eliminated *Fusobacterium* and *Prevotella* species, as well as beta-hemolytic *Streptococcus*, in ligature-induced peri-implantitis in dogs (86, 181). Similarly, in a clinical study, photodynamic therapy achieved significant, but incomplete, elimination of *Aggregatibacter actinomycetemcomitans* (previously *Actinobacillus actinomycetemcomitans*), *Porphyromonas gingivalis* and *Prevotella intermedia* (49). It was also reported that photodynamic therapy, in combination with guided bone regeneration, produced bone defect fill and re-osseintegration (179) and greater bone gain than mechanical biofilm removal from the implants and guided bone regeneration (180) in ligature-induced peri-implantitis in dogs. In a clinical study, the combination of toluidine blue O-mediated photodynamic therapy with guided bone regeneration resulted in the reduction of bone defects (the mean radiographic peri-implant bone gain was 2 mm) in 21 of 24 implants at 9.5 months following treatment (80). Figure 7 illustrates the use of methylene blue-mediated photodynamic therapy as an adjunct to the surgical treatment of peri-implantitis in a clinical setting.

A variety of experimental studies are needed to evaluate photodynamic therapy in the treatment of peri-implantitis. One *in vitro* photodynamic therapy study has demonstrated the complete elimination of *A. actinomycetemcomitans*, *P. gingivalis* and *P. intermedia* on titanium plates (81). Future studies should seek to reproduce these observations and establish optimal photosensitizer and light parameters for targeting multispecies microbial biofilms on dental implants *in vitro*. These studies should explore the photodynamic therapy effects alone compared with chemical treatments. The role of photodynamic therapy as an adjunctive methodology to nonsurgical therapy has considerable promise.

Endodontic infection

The ultimate goal of endodontic treatment is the elimination of infection from the root canal system. This principle is supported by studies that demonstrate significantly higher success rates (of 10% or more) in teeth that are minimally infected at the time of treatment, compared to grossly infected teeth with necrotic pulps (186). Similarly, teeth which give a negative culture for bacterial growth at the time of root canal filling have a higher success rate (12–26% higher) than teeth that are culture positive (56, 185). More than 20 million root canal treatment procedures are performed yearly in the USA and more than 2 million endodontic retreatments are required. Retreatment often involves surgery that could be avoided in many cases by better disinfection procedures (21). Therefore, dental root canal disinfection is critical to success. By contrast, the bacterial microflora of primary endodontic infection differs from that of post-treatment endodontic disease. Both culture methods and polymerase chain reaction-based methods have demonstrated that primary endodontic infections are associated with polymicrobial and strictly anaerobic microorganisms (170, 183, 184). Endodontic treatment failures, however, are frequently associated with gram-positive aerobic and facultative microorganisms (183). The presence of *Enterococcus faecalis* in failed endodontic treatment has been extensively reviewed in the literature (84, 201) but this species of bacterium is rarely detected in primary infected and untreated cases. Yet, one cannot discount the presence or significance of other microorganisms belonging to the genera *Actinomyces* and *Propionibacterium*, which have been frequently detected in endodontic treatment failures (84, 159, 201). The current treatment procedures to eliminate infection include mechanical removal of the infected contents of the canal system, irrigation with an antibacterial/tissue-dissolving agent (usually sodium hypochlorite), inter-appointment dressing of the canal with calcium hydroxide (which has modest antibacterial activity) and obturation of the root canal space. The complexity, however, of the root canal system with its isthmuses, ramifications, as well as the presence of dentinal tubules, make complete debridement and removal of bacteria with instrumentation, irrigation and the standard medicaments almost impossible. In addition, current endodontic procedures require very good technical skills and use medicaments whose effectiveness has never been definitively proven in human clinical trials. Therefore, the need for better root canal disinfection is clear and compelling.

Photodynamic therapy has been employed in recent years to target microorganisms in root canals *in vitro* (7, 61, 63, 65, 68, 70–73, 116, 177, 182, 190, 219) and *in vivo* (17, 18, 69, 160). These studies suggested the potential of photodynamic therapy as an adjunctive technique to eliminate residual root canal bacteria after standard endodontic chemo-mechanical debridement. Methylene blue has been used as the photosensitizer for targeting endodontic microorganisms in several studies (61, 65, 71–73, 116, 182).

The synergism of red light and methylene blue reduced the viability of *E. faecalis* in the root canals of experimentally infected teeth by 40% (182). In other studies, the combined effect of methylene blue and red light at 665 nm resulted in the reduction of *E. faecalis* viability by 78–97% in the root canals of experimentally infected human teeth (65, 190). The photodynamic effects of methylene blue were also investigated on multispecies root canal biofilms comprising four species of microorganisms in experimentally infected root canals of extracted human teeth (61). Photodynamic therapy achieved a reduction in bacterial viability of up to 80%. The results of this study suggested the potential of photodynamic therapy to be used as an adjunctive antimicrobial procedure after standard endodontic chemo-mechanical debridement, but also demonstrated the importance of further optimization of light dosimetry for bacterial photodestruction in root canals. Methylene blue dissolved in a mixture of glycerol:ethanol:water (71, 72), as well as a methylene blue formulation containing an emulsion of oxidizer:oxygen carrier (73), enhanced the photodynamic effects of methylene blue *in vitro*. Findings from a recent study showed the efficacy of photodynamic therapy mediated by methylene blue dissolved in a mixture of glycerol:ethanol:water in the presence of an irradiation medium (perfluorodecahydro-napthalene) to eradicate *E. faecalis* biofilms in the root canal system of experimentally infected human teeth (116). Methodologic differences in all of these *in vitro* studies that employed photodynamic therapy for targeting root canal microorganisms make comparisons difficult. Those studies have used different PS, such as toluidine blue O (7, 63, 160, 177, 219), azulene (68) and chlorin e6 (69, 70), as well as different light parameters and light-delivery techniques. Recently, two studies described the application of root canal photodynamic therapy *in vivo* using toluidine blue O and light (17, 18). It was suggested that photodynamic therapy offered a means of destroying microorganisms remaining after using sodium hypochlorite alone (17) or citric acid and sodium hypochlorite as co-irrigants (18).

When a photoactive compound is applied in the root canal system, it will have access to residual bacteria in the main canals, isthmuses, lateral canals and dentinal tubules. It is also possible that this compound may escape into the periapical tissues. During photodynamic therapy, light will excite the drug in bacteria within the root canal, but could also potentially affect the periapical host cells that have taken up the drug. Therefore, it is important to establish the safety of photodynamic therapy, and to determine the therapeutic window whereby bacteria can be eliminated but host cells are left intact. The safety of photodynamic therapy for endodontic disinfection has been addressed in two recent *in vitro* studies (72, 231). The photodynamic effects of methylene blue dissolved in water were tested on fibroblasts and *E. faecalis* (72). Concentrations of methylene blue ranging from 10 to 100 μM produced up to 36 and 100% killing for fibroblasts and *E. faecalis*, respectively, after incubation for 20 min followed by exposure to red light with a total energy of 36 J (72). We assessed the viability of human gingival fibroblasts and osteoblasts *in vitro* after exposure to methylene blue and red light with parameters similar to those that may be applied in a clinical setting (231). A 250-μm-diameter polymethyl methacrylate optical fiber was used that uniformly distributed light over 360° (Fig. 8). With this fiber, <10% of light energy delivered in the root canal system escapes from the root apex. Although the power density of light within the root canal is 100 mW/cm^2 (61), that in the periapical region ranges from 5 to 10 mW/cm^2, depending on the anatomy of the root canal. Both cell types were sensitized with 50 μg/ml of methylene blue followed by exposure to red light at 665 nm for 5 min with an irradiance of 10, 20 and 40 mW/cm^2. Light at 20 and 40 mW/cm^2 with methylene blue had modest effects at 24 h on osteoblasts in both assays, whereas the use of sodium hypochlorite

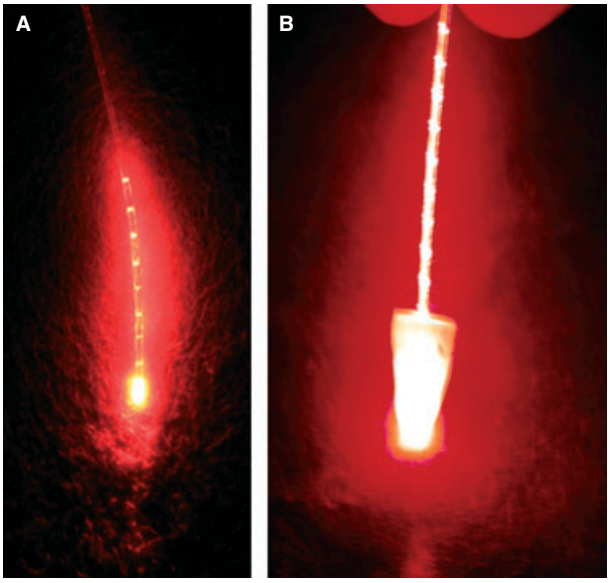


Fig. 8. Optical fiber with a diameter of 250 μm for uniform illumination (A) (61). Exposure of the root canal system of a tooth specimen to red light (B).

completely eliminated cells. Western blot analysis revealed no signs of apoptosis in either cell type. Photodynamic therapy, as an adjunctive technique to standard endodontic treatment, may have potential in the clinical setting by providing a large therapeutic window whereby residual root canal bacteria can be killed without harming cells in the periapical region.

Photodynamic therapy has been used for endodontic disinfection in a clinical setting as an adjunct to standard endodontic treatment (17, 18, 69, 159). In these studies, the application of photodynamic therapy was rapid. Following the completion of standard endodontic treatment, the photosensitizer was applied in the root canal system for 1–3 min. Then, a fiberoptic was used to deliver light from a diode laser to irradiate the root canal system for 2–4 min. Bonsor et al. (17, 18) studied the microbiological effects of photodynamic therapy on root canal bacteria following the use of conventional irrigants. In these studies, the photo-activated disinfection system, PAD™ (Denfotex Light Systems Ltd, Inverkeithing, UK), was used. The PAD™ consists of toluidine blue O solution and a 100 mW diode laser that emits light at 635 nm. Toluidine blue O (12.7 mg/l) was applied in the root canals for 60 s followed by exposure to light via a fiberoptic for 2 min. Photodynamic therapy was able to rapidly eliminate microorganisms, whereas conventional therapy was unable to do so. In another study (159), the synergism of chemical-mechanical instrumentation and toluidine blue O-mediated photodynamic therapy reduced the bacterial numbers by 98.37%, whereas chemical-mechanical instrumentation alone reduced the bacterial numbers by 82.59%. Similar data were obtained in a study, in which a conjugate between polyethyleneimine and the photosensitizer, chlorine 6, was used for targeting root canal microorganisms (69).

Photodynamic therapy shows great promise for application in the field of endodontics. Of particular significance here, is the rapidity with which an effect can be generated. Future experimental studies should explore the use of novel technologies for increased delivery of methylene blue or toluidine blue O in dentinal tubules and the application of supplemental hyperoxygenation in the root canal system to enhanceme the photodynamic therapy effect. The assessment of the efficacy of dentinal tubule disinfection following standard endodontic treatment and photodynamic therapy *ex vivo*, using freshly extracted infected teeth, would be instructive before clinical studies are conducted.

Oral candidiasis

Candida albicans becomes a serious opportunistic infectious agent in immunocompromised patients (165). *C. albicans* can grow as biofilms on oral mucosal surfaces (92) and prosthetic devices (98). Antifungal treatment with agents such as nistatin and miconazole often induce resistance, severely limiting their ability to eradicate fungal biofilms, so that recurrent infection occurs (91). Numerous *in vitro* studies have shown that photodynamic therapy is effective in killing *Candida* in planktonic (16, 35, 74, 143, 187, 196, 197, 228) and biofilm (30, 48) phases using methylene blue (74, 143, 196, 197, 228), toluidine blue O (48, 196, 228), photofrin (30), tionin (228), porphyrins (35), phthalocyanine (187, 228) and malachite green (196). Recently, Soares et al. (188) showed that toluidine blue O-mediated photodynamic therapy eliminated different *Candida* isolates and also inhibited their adhesion to buccal epithelial cells *in vitro*. Short application times of toluidine blue O-containing mucoadhesive patches, followed by exposure to light, allowed killing of *C. albicans* in suspension, but not in biofilms (48). Teichert et al. (205) investigated the effects of methylene blue-mediated photodynamic therapy on buccal candidiasis in immunosuppressed mice. The authors were able to show a dose-dependent photodestruction curve that led to complete elimination of *C. albicans* at concentrations ranging from 450 to 500 μg/ml. In another study, rats with experimentally induced buccal candidiasis that were exposed to methylene blue-mediated photodynamic therapy exhibited fewer epithelial alterations and a lower chronic inflammatory response (95).

Topical treatment of oral candidiasis by photodynamic therapy may be an alternative to traditional antifungal drug therapy, especially in patients with human immunodeficiency virus (HIV) for whom persistent infection is a major problem (28). Further animal studies should establish a protocol for successful targeting of candidiasis lesions, which will then be tested in human studies. Recently, it has been shown that laser irradiation alone exerted antifungal effects *in vitro* (196, 197). These data are supported by a human study, in which a reduction of inflammation was observed on the palate of subjects with denture stomatitis after five consecutive treatments with laser irradiation (129). The presence of endogenous chromophores within *C. albicans* that may contribute to photosensitization requires further investigation.

New frontiers in oral antimicrobial photodynamic therapy

The role of photodynamic therapy as a local treatment of oral infection, either in combination with traditional methods of oral care, or alone, arises as a simple, nontoxic and inexpensive modality with little risk of microbial resistance. Lack of reliable clinical evidence, however, has not allowed the effectiveness of photodynamic therapy to be confirmed. Studies have been performed using different treatment conditions and parameters with insufficient clinical and microbiological findings. The reduced susceptibility of complex oral biofilms to antimicrobial photodynamic therapy may require the development of novel delivery and targeting approaches. Evolving therapeutic strategies for biofilm-related infections include the use of substances designed to target the biofilm matrix, nongrowing bacteria (persister cells) within biofilms and/or quorum sensing (46). The use of bacteriophages (29) and naturally occurring or synthetic antimicrobial peptides (173) may offer the possibility of bacterial targeting without the emergence of resistance. Recently, the advantages of targeted therapy become more apparent, and the use of light alone, antibody–photosensitizer and bacteriophage–photosensitizer conjugates or nonantibody-based targeting moieties, such as nanoparticles, are gaining increasing attention.

Phototherapy

In some instances, application of a photosensitizer may not be required because photosensitizers occur naturally within some microbial species. This is particularly true of the oral black-pigmented species. We have shown that broadband light ranging from 380 to 520 nm was able to achieve a threefold reduction in the growth of *P. gingivalis*, *P. intermedia*, *Prevotella nigrescens* and *Prevotella melaninogenica* in dental plaque samples obtained from human subjects with chronic periodontitis (193). In this study, the presence and amounts of endogenous porphyrins in black-pigmented bacteria were estimated (Fig. 9) and analysis of bacteria in dental plaque samples was performed by DNA–DNA hybridization for 40 taxa before and after phototherapy (Fig. 10). Inactivation of black-pigmented bacteria by visible light has also been reported by other investigators (60, 66, 87, 88, 104, 193, 198).

Black-pigmented bacteria, such as *P. intermedia, P. nigrescens* and *P. melaninogenica*, are associated with gingivitis (38, 78, 202) and may be responsible for the increased bleeding tendency of long-standing gingivitis (78). *Prevotella* species have also been recognized as potent producers of volatile sulfur compounds on the dorsum of the tongue (144) and were detected at high numbers in tongue samples obtained from subjects with oral malodor (209, 217). In another study, human salivary microflora was exposed to blue light of 400–500 nm and a reduction in the levels of volatile sulfide compounds was found, together with a selective inhibitory effect on the gram-negative bacteria, suggesting that it may be possible to use light to treat oral malodor (198). Additionally, black-pigmented bacteria, such as *P. gingivalis* and *P. intermedia*, are associated with the development of periodontitis (140, 189), which is thought to be involved in the pathogenesis of cardiovascular disease (134); black-pigmented bacteria were detected in atheroma plaques (32, 85, 203) and

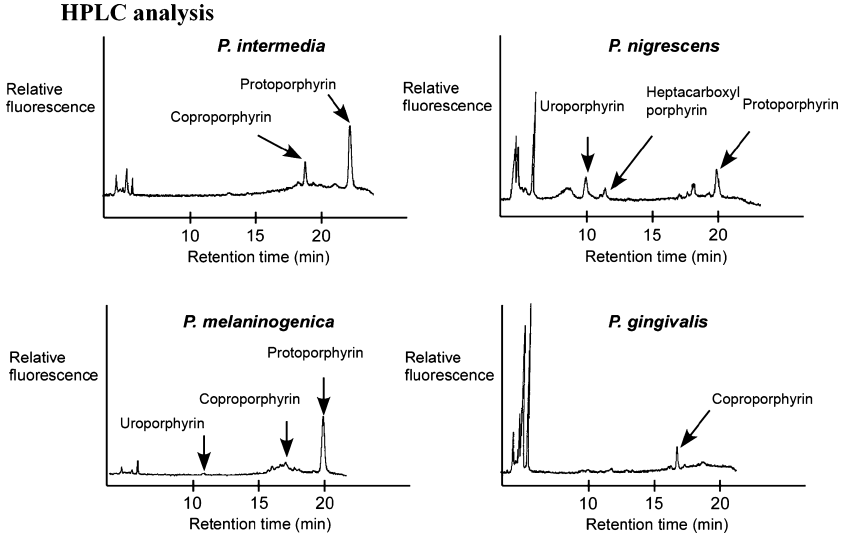
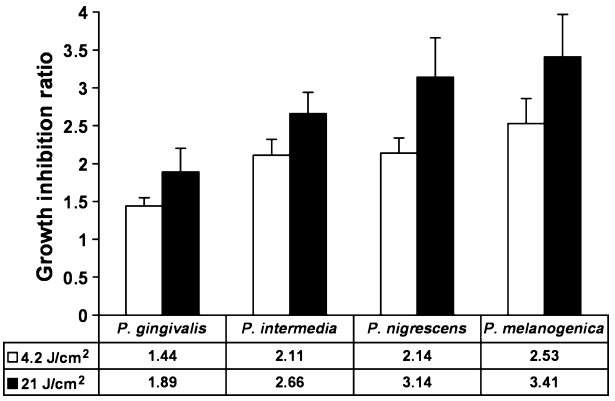


Fig. 9. High-performance liquid chromatography (HPLC) analysis of the porphyrin content of oral black-pigmented bacteria (193).



	P. gingivalis	P. intermedia	P. nigrescens	P. melanogenica
□4.2 J/cm²	1.44	2.11	2.14	2.53
■21 J/cm²	1.89	2.66	3.14	3.41

Fig. 10. Inhibition of the growth of black-pigmented bacteria after exposure to light with energy fluencies of 4.2 and 21 J / cm^2. The bars represent the ratio of DNA counts before exposure to light to the DNA counts after exposure to light (mean ± standard error of the mean, 15 subjects). The order of growth inhibition was *Prevotella melaninogenica* > *Prevotella nigrescens* > *Prevotella intermedia* > *Porphyromonas gingivalis* for both energy fluencies (193).

their presence in subgingival plaque samples was positively associated with elevated C-reactive protein levels (146).

Based on these observations, we can propose an intra-oral phototherapeutic strategy. Consider that in healthy subjects, dental plaque remains stable for prolonged periods of time because of a dynamic balance among the resident members of its microbial community (126). An increase in the number of pathogens in the microbial community is caused by the breakdown of the microbial homeostasis induced by the disturbance of the local habitat (125). In this case, specific suppression of key pathogens may result in an increase in the microbial flora that is associated with oral health.

The above studies introduce new research paths, where visible light could be used prophylactically. Daily and very short exposures of periodontal pockets, and of the mucosa of the dorsum of the tongue, to visible light (mainly blue light) in human subjects with gingivitis, periodontitis and oral malodor may lead to a cumulative suppressive effect on both dental plaque and tongue black-pigmented species by activating their endogenous porphyrins. This may have an impact on the reduction of bleeding in gingivitis, the reduction of inflammation in periodontitis and the cure of oral malodor. In all of the cases, exposure to visible light may result in the gradual suppression of black-pigmented bacteria that will lead to a shift of the microbial composition towards a new one associated with health. This novel technique may offer the following advantages compared with other forms of periodontal therapy (scaling, mouthwashes and surgery): (i) rapid and painless application of light; (ii) selectivity in its effect; (iii) full penetration of dental plaque by light; (iv) limited penetration of light into gum tissue; (v) absence of phototoxicity to human cells; (vi) no effects on taste; and (vii) possible clinical and microbiological benefit with minimal impact on natural microbiota.

Antibody-targeted antibacterial approaches using photodynamic therapy

Antibodies conjugated with photosensitizers have been used to target *Staphylococcus aureus* (53, 54, 79). Selective killing of *P. gingivalis* was achieved in the presence of *Streptococcus sanguinis* (previously *S. sanguis*) or in human gingival fibroblasts using a murine monoclonal antibody against *P. gingivalis* lipopolysaccharide conjugated with toluidine blue O (13). In two studies, bacteriophages were used as vehicles to deliver the photosensitizer tin(IV) chlorine e6 to the surface of *S. aureus* strains (55, 89). This led to approximately 99.7% killing of microorganisms (89). The combination of pulsed laser energy and absorbing gold nanoparticles selectively attached to the bacterium for killing of microorganisms is a new technology that was introduced recently (236). Gold nanoparticles are promising candidates for application as photothermal sensitizers and can easily be conjugated to antibodies. The surface of *S. aureus* was targeted using 10- to 40-nm gold nanoparticles conjugated with anti-protein antibodies (236). The energy that was absorbed by nanoparticles during irradiation was quickly transferred through nonradiative relaxation into heat accompanied by bubble-formation phenomena around clustered nanoparticles, leading to irreparable bacterial damage.

Antibody-targeted approaches using photodynamic therapy have been most frequently focused on the treatment of malignant diseases. The therapeutic potential of these approaches for bacterial targeting is based on their ability to demonstrate minimal damage to host cells. Therefore, these approaches should be further explored *in vitro* and in animal studies.

Nanoparticle-based antimicrobial photodynamic therapy

Incomplete penetration of methylene blue in oral biofilms may become greater in a clinical setting, where both the photoactive compound and light should be applied for periods of up to 15 min. Therefore, one of the ways to overcome these

deficiencies is to develop delivery systems that significantly improve the pharmacological characteristics of methylene blue. Recently, we proposed the encapsulation of methylene blue within poly(D,L-lactide-co-glycolide) (PLGA) nanoparticles (~150–200 nm in diameter) that may offer a novel design of nano-platform for enhanced drug delivery and photodestruction of oral biofilms (155). Engineered biodegradable polymeric nanoparticles made of PLGA (110) have been used as a drug-delivery system for various photosensitizers (77, 101, 103, 130, 169, 211, 235). The nanoparticle matrix PLGA is a polyester co-polymer of polylactide and polyglycolide that has received approval by the US Food and Drug Administration as a result of its biocompatibility and its ability to degrade in the body through natural pathways (157). Once encapsulated within PLGA, the excited state of the PS is quenched, which results in the loss of phototoxicity. When the nanoparticles were incubated with cells, they showed a time-dependent release of the PS, which then regained its phototoxicity and resulted in an activatable photodynamic therapy-nanoagent (130). In our studies, sensitization of *E. faecalis* species in planktonic phase with methylene blue-loaded nanoparticles for 10 min, followed by exposure to red light at 665 nm, led to approximately 99% bacterial killing, whereas the synergism of nanoparticles and light exhibited approximately 10-fold killing of *E. faecalis* biofilm species in experimentally infected root canals of human extracted teeth (155). The uptake and distribution of nanoparticles in *E. faecalis* in suspension was investigated by transmission electron microscopy after incubation with PLGA complexed with colloidal gold particles for 2.5, 5 and 10 min. Nanoparticles were not internalized by microorganisms, but they were mainly concentrated onto their cell walls (Fig. 11). This may have rendered the cell wall permeable to methylene blue released by the nanoparticles. In this case, the intracellular localization and the local surroundings of methylene blue influence the phototoxicity. It is also possible that photodestruction takes place within the cell wall. In this case the intracellular content may have leaked out. However, the fact that methylene blue-loaded nanoparticles alone reduced bacterial survival by 34 to 58.5% suggests that methylene blue penetrated the bacterial cell well.

The use of biodegradable polymer to synthesize the nanoparticles makes the final product attractive for clinical use. This nanoagent has several favorable properties for use as a photosensitizer (105): (i) a large critical mass (concentrated package of photosensitizer) for the production of reactive oxygen

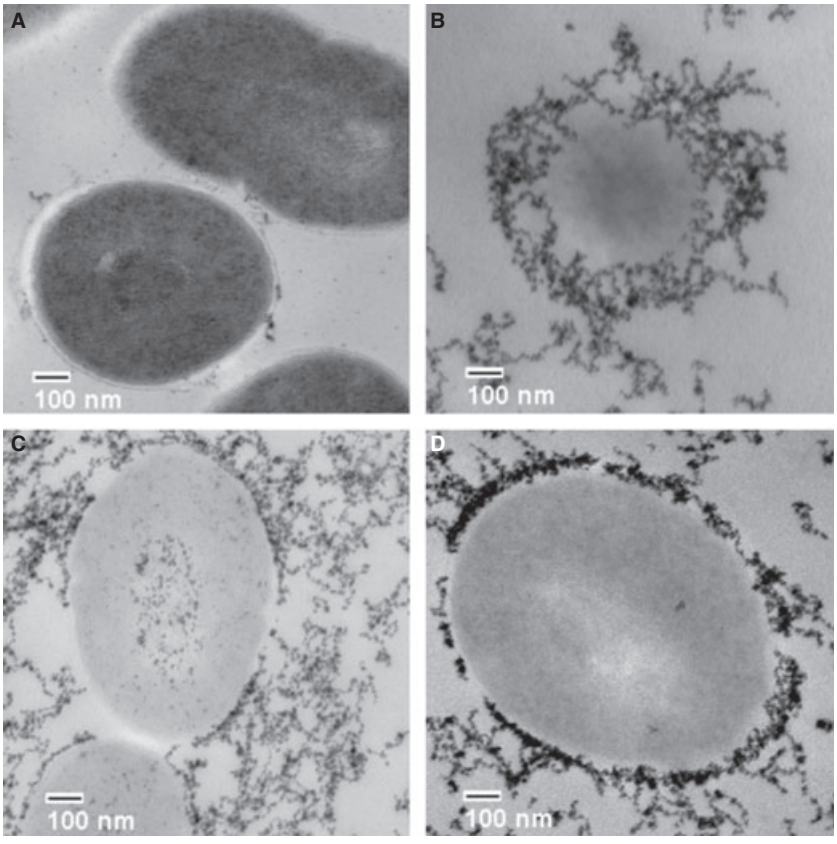


Fig. 11. Transmission electron microscopy of *Enterococcus faecalis* (A). Colloidal gold particles complexed with poly(lactic-*co*-glycolic acid) are concentrated mainly on the cell walls of microorganisms after 2.5 min (B), 5 min (C) and 10 min (D) of incubation. Complexing gold nanoparticles with poly(D,L-lactide-co-glycolide) (PLGA) showed a high contrast that could not be provided by methylene blue-loaded PLGA nanoparticles. The surface properties (size and charge) of nanoparticles were the same for gold as they were for methylene blue (155).

species that destroy cells; (ii) it limits the cell's ability to pump the drug molecule back out and reduces the possibility of multiple drug resistance; (iii) selectivity of treatment by localized delivery agents, which can be achieved by either passive targeting or by active targeting via the charged surface of the nanoparticle; and (iv) the nanoparticle matrix is nonimmunogenic.

PLGA nanoparticles loaded with various compounds (e.g. antibiotics) have been used for bacterial targeting (1, 57, 93, 97, 111, 156); however, the use of PLGA nanoparticles as carriers of photosensitizers has not been explored in antimicrobial photodynamic therapy until recently. In future, a more thorough evaluation of the photodynamic effects of methylene blue-loaded nanoparticles would also require knowledge of various parameters that would lead to a maximum photodynamic effect on oral microbes, such as: the amount of methylene blue encapsulated in nanoparticles; the incubation time of methylene blue-loaded nanoparticles with microorganisms; the power density (mW/cm^2); and the energy fluence (J/cm^2) of light. In addition, the therapeutic window where microorganisms would be killed by methylene blue-loaded nanoparticles while leaving normal cells intact, as well as the role of nanoparticle charge, should also be explored. At a later stage, a comparison between the photodynamic effects of methylene blue-loaded nanoparticles and free methylene blue would be necessary.

Conclusions

The potential applications of photodynamic therapy to treat oral conditions seem limited only by our imagination. Applications appear not only the common oral diseases of dental caries and periodontal disease but also the conditions of oral cancer, periimplantitis, endodontic therapy, candidiasis and halitosis. Low toxicity and rapidity of effect are qualities of photodynamic therapy that are enviable. It is now the time to demonstrate clear evidence of clinical efficacy and applicability. At this time in history, it is difficult to know where light will lead us in the oral cavity but the promise is clear and the opportunities are visible.

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