CRITICAL REVIEWS IN ORAL BIOLOGY & MEDICINE

Photodynamic Therapy in Dentistry

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ABSTRACT

Photodynamic therapy (PDT), also known as photoradiation therapy, phototherapy, or photochemotherapy, involves the use of a photoactive dye (photosensitizer) that is activated by exposure to light of a specific wavelength in the presence of oxygen. The transfer of energy from the activated photosensitizer to available oxygen results in the formation of toxic oxygen species, such as singlet oxygen and free radicals. These very reactive chemical species can damage proteins, lipids, nucleic acids, and other cellular components. Applications of PDT in dentistry are growing rapidly: the treatment of oral cancer, bacterial and fungal infection therapies, and the photodynamic diagnosis (PDD) of the malignant transformation of oral lesions. PDT has shown potential in the treatment of oral leukoplakia, oral lichen planus, and head and neck cancer. Photodynamic antimicrobial chemotherapy (PACT) has been efficacious in the treatment of bacterial, fungal, parasitic, and viral infections. The absence of genotoxic and mutagenic effects of PDT is an important factor for long-term safety during treatment. PDT also represents a novel therapeutic approach in the management of oral biofilms. Disruption of plaque structure has important consequences for homeostasis within the biofilm. Studies are now leading toward selective photosensitizers, since killing the entire flora leaves patients open to opportunistic infections. Dentists deal with oral infections on a regular basis. The oral cavity is especially suitable for PACT, because it is relatively accessible to illumination.

KEY WORDS: photosensitizers, photodynamic therapy, head and neck cancer, cancer therapy, photodynamic antimicrobial chemotherapy.

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(1) INTRODUCTION

Photodynamic therapy (PDT) is a medical treatment that utilizes light to activate a photosensitizing agent (photosensitizer) in the presence of oxygen. The exposure of the photosensitizer to light results in the formation of oxygen species, such as singlet oxygen and free radicals, causing localized photodamage and cell death. Clinically, this reaction is cytotoxic and vasculotoxic. Depending on the type of agent, photosensitizers may be injected intravenously, ingested orally, or applied topically. The relative simplicity of the mechanism of activation of photosensitizers has stimulated considerable interest in PDT. Advantages of PDT over the conventional treatments of cancer, such as surgery, radiotherapy, and chemotherapy, are summarized in Table 1 (Hopper, 1996, 2000; Dougherty *et al.*, 1998; Brown *et al.*, 2004; Allison *et al.*, 2006). PDT has been approved for clinical treatment in the United States, the European Union, Canada, Russia, and Japan (Biel, 2002, 2006; Allison *et al.*, 2004c; Kübler, 2005). Currently, PDT is being applied mostly in the treatment of cancer (Hopper, 2000; Biel, 2002, 2006; Allison *et al.*, 2004c, 2005, 2006); however, several studies have shown that PDT also has antimicrobial properties (Wainwright, 1998; Hamblin and Hasan, 2004; Meisel and Kocher, 2005; O'Riordan *et al.*, 2005; Smith, 2005; Kömerik and MacRobert, 2006; Wood *et al.*, 2006; Donnelly *et al.*, 2007). Photodynamic antimicrobial chemotherapy (PACT) represents an alternative antibacterial, antifungal, and antiviral treatment for drug-resistant organisms (Wainwright and Crossley, 2004). It is unlikely that bacteria would develop resistance to the cytotoxic action of singlet oxygen or free radicals. Bacteria that grow in biofilms, implicated in diseases like cystic fibrosis (*Pseudomonas aeruginosa*) or periodontitis (*Porphyromonas gingivalis*), are also susceptible to PDT (Bhatti *et al.*, 1998; Wood *et al.*, 1999). Applications of PDT in dentistry are growing rapidly: the treatment of oral cancer, as well as bacterial and fungal infections, and the photodynamic diagnosis (PDD) of the malignant transformation of oral lesions (Sharwani *et al.*, 2006). The non-oncological applications of PDT include treatment of psoriasis (Weinstein *et al.*, 1991), actinic keratosis (Itoh *et al.*, 2000), rheumatoid arthritis (Miyazawa *et al.*, 2006), and age-related macular degeneration (Kozak *et al.*, 2006). The aim of this review is to outline the clinical results of PDT for the treatment of head and neck cancer and of PACT for the treatment of oral infections.

(1.1) Photodynamic Reaction

PDT involves three components: light, a photosensitizer, and oxygen. A photosensitizer or its metabolic precursor is administered to the patient. Upon irradiation with light of a specific wavelength, the photosensitizer undergoes a transition from a low-energy ground state to an excited singlet state. Subsequently, the photosensitizer may decay back to its ground state, with emission of fluorescence, or may undergo a transition to a higher-energy triplet state. The triplet state can react with endogenous oxygen to produce singlet oxygen and other radical species, causing a rapid and selective destruction of the target tissue (Fig. 1). There are two mechanisms by which the triplet-state photosensitizer can react with biomolecules. Type I involves electron/hydrogen transfer directly

Table 1. Potential Advantages of Photodynamic Therapy over Conventional Anti-cancer Therapies

- Is non-invasive and convenient for the patient
- Can be performed in outpatient or day-case (inpatient) settings
- Can be targeted accurately and selectively in early or localized diseases
- Although it cannot cure advanced disseminated disease, because illumination of the whole body is not possible, it can improve quality of life and lengthen survival
- Repeated doses can be given without the need for total-dose limitations
- Has moderate side-effects
- Can have excellent cosmetic results, and the healing process results in little or no scarring
- Can offer organ-sparing treatment worldwide, with very little investment in infrastructure

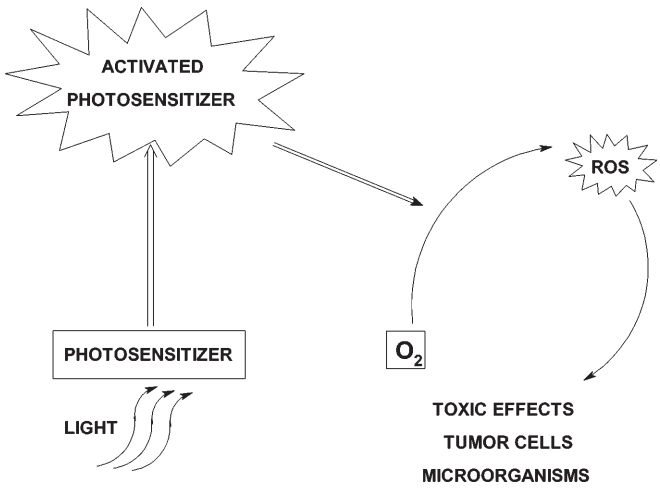

Figure 1. Schematic representation of photodynamic reaction and photodynamic therapy. Light (photon) of an appropriate energy (*e.g.*, with wavelength at the absorption maximum) is absorbed by a photosensitizer, which undergoes a transition from a low-energy ground state to the excited-singlet state. The activated photosensitizer interacts with oxygen to produce singlet oxygen and other radical species that cause a toxic effect in tumor cells or micro-organisms; ROS, reactive oxygen species.

from the photosensitizer, producing ions, or electron/hydrogen removal from a substrate molecule to form free radicals. These radicals react rapidly with oxygen, resulting in the production of highly reactive oxygen species (superoxide, hydroxyl radicals, hydrogen peroxide). Type II reactions produce the electronically excited and highly reactive state of oxygen known as singlet oxygen. In PDT, it is difficult to distinguish between the two reaction mechanisms. A contribution from both Types I and II processes indicates that the mechanism of damage is dependent on both oxygen tension and photosensitizer concentration.

PDT-mediated tumor destruction *in vivo* involves cellular, vascular, and immunological mechanisms. The relative contribution of each depends on the type of photosensitizer and its localization inside the tumor, vascularity of the tumor, and the drug-to-light interval. Usually, after injection, a photosensitizer is initially detained within the tumor vasculature, and PDT, which utilizes a short drug-to-light interval, damages mainly the tumor vasculature. A long drug-to-light interval allows for diffusion of the photosensitizer into the tissue, its accumulation into cellular compartments, and more direct tumor cytotoxicity. The effectiveness of cellular targeting of the photosensitizer is also affected by heterogenous perfusion, vascular permeability, and interstitial pressure of the tumor (Chen B *et al.*, 2005; Solban *et al.*, 2006).

PDT produces cytotoxic effects through photodamage to subcellular organelles and molecules. Mitochondria, lysosomes, cell membranes, and nuclei of tumor cells are considered potential targets, along with the tumor vasculature. During light exposure, sensitizers that localize in mitochondria may induce apoptosis, while sensitizers localized in lysosomes and cell membranes may cause necrosis (Castano *et al.*, 2005). The apoptotic effect of PDT provides a rationale for the widespread efficacy of PDT in different tumors. The *in vivo* tumoricidal reaction after PDT is accompanied by complex inflammatory and immune responses (Dougherty *et al.*, 1998). A massive invasion of neutrophils, mast cells, and monocytes/macrophages during and after PDT has been observed in murine tumor models (Krosl *et al.*, 1995). This can be followed by an activation of specific T-lymphocytes (Nowis *et al.*, 2005). After illumination of the photosensitizer, an acute stress response leads to changes in calcium and lipid metabolism, and production of cytokines and stress proteins. Subcellular and tumor localization of photosensitizers, and molecular, cellular, and tumor responses associated with PDT have been reviewed by Dougherty *et al.* (1998).

PDT generates measurable changes in tumor oxygen and blood flow during illumination. Damage to the tumor vascular network can diminish the supply of oxygen to the tumor. In addition, the production of reactive oxygen species is associated with utilization of oxygen. This process, known as photochemical oxygen consumption, can also generate hypoxia during treatment (Busch, 2006). Since oxygen is required for PDT, the illumination-induced hypoxia can further reduce the tumor response. The development of sensitive methods of quantifying tumor oxygen and evaluating its distribution in tissues can improve the treatment protocols.

(1.2) Light Sources

PDT requires a source of light that activates the photosensitizer by exposure to low-power visible light at a specific wavelength. Human tissue transmits red light efficiently, and the longer activation wavelength of the photosensitizer results in deeper light penetration. Consequently, most photosensitizers are activated by red light between 630 and 700 nm, corresponding to a light penetration depth from 0.5 cm (at 630 nm) to 1.5 cm (at ~ 700 nm) (Salva, 2002; Kübler, 2005). This limits the depth of necrosis and/or apoptosis and defines the therapeutic effect. As a result, larger solid tumors cannot be uniformly illuminated, because of the limited depth of light penetration. The total light dose, the dose rates, and the depth of destruction vary with each tissue treated and with each photosensitizer (Grant *et al.*, 1997; Biel, 2002; Allison *et al.*, 2005, 2006).

In the past, photosensitizer activation was achieved *via* a variety of light sources, such as argon-pumped dye lasers, potassium titanyl phosphate (KTP)- or neodymium:yttrium aluminum garnet (Nd/YAG)-pumped dye lasers, and gold

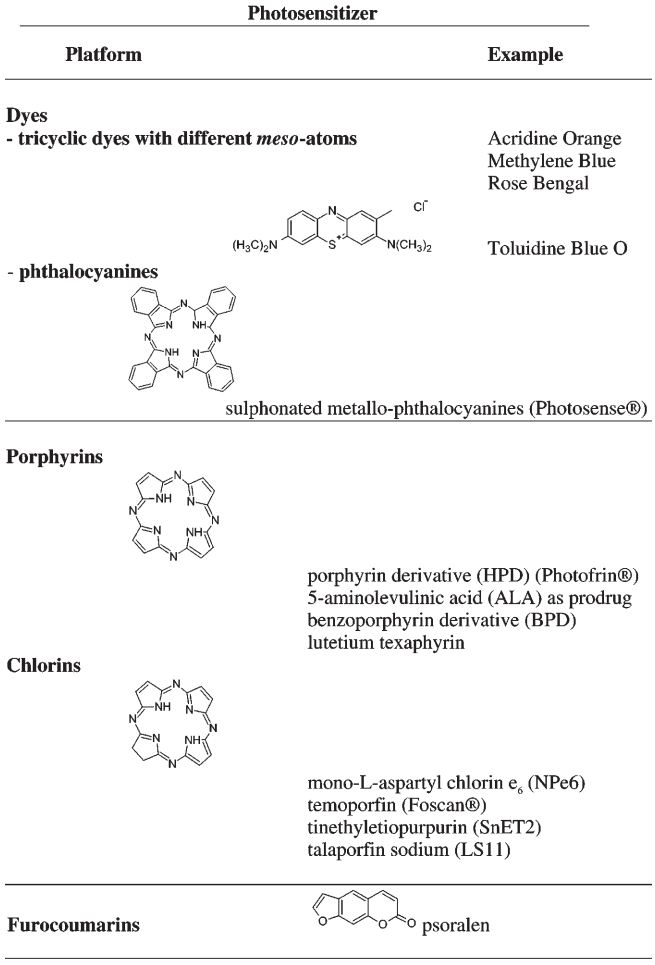

Figure 2. Photosensitizers used in photodynamic therapy.

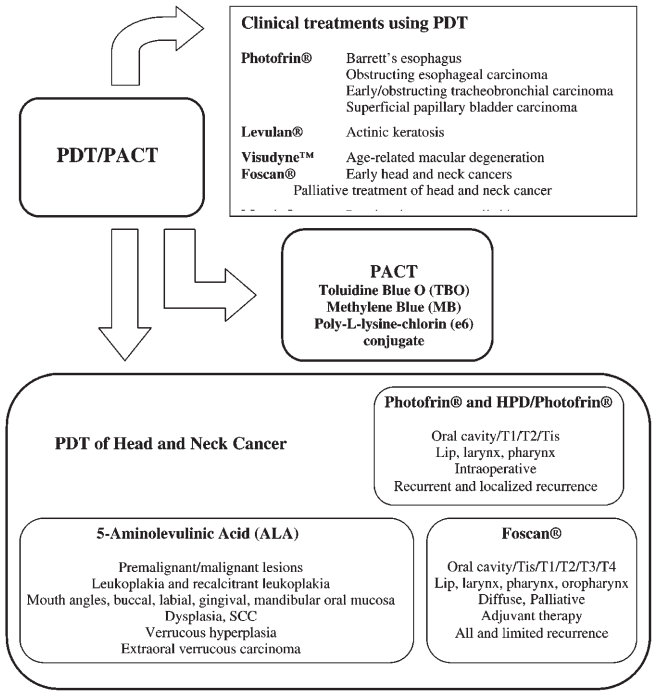

Figure 3. Photosensitizers used in the different clinical applications of photodynamic therapy (PDT) and photodynamic antimicrobial chemotherapy (PACT).

vapor- or copper vapor-pumped dye lasers. All these laser systems are complex and expensive. At present, diode laser systems that are easy to handle, portable, and cost-effective are used predominantly (Kübler, 2005). For treatment of larger areas, non-coherent light sources, such as tungsten filament, quartz halogen, xenon arc, metal halide, and phosphor-coated sodium lamps, are in use. Recently, non-laser light sources, such as light-emitting diodes (LED), have also been applied in PDT (Allison *et al.*, 2004c; Juzeniene *et al.*, 2004; Pieslinger *et al.*, 2006; Steiner, 2006). These light sources are much less expensive and are small, lightweight, and highly flexible.

Different techniques are used to illuminate the tumor. These include superficial, interstitial, intra-operative, and intra-cavitary PDT. Interstitial light delivery is appropriate for tumors in which surgery would involve extensive resection, or for those that are not suitable for surgery. In intra-operative PDT, which is used as an adjuvant treatment in anatomically complex areas, the photosensitizer is applied to the patient several days prior to the operation, and resection of the tumor is followed by photosensitizer activation (Kübler, 2005). Sources used for light delivery in PDT vary, depending upon the location and morphology of the lesion, but are typically fiber-optic catheters terminated with cylindrical diffusers or lenses for flat-field applications (Biel, 2002; Mang, 2004; Allison *et al.*, 2005). The light field produced should be uniform, allowing for a precise calculation of the delivered dose. The tip of the fiber can be made into various shapes, allowing for diffusion in all directions or for focus, as with a flashlight (diffusing type). Unfortunately, different fibers are not always available, are very expensive, and are not FDA-approved, except for diffusers. Thus, diffusing fibers (1-5 cm) are generally the only ones commercially available, while fiber-optic systems that transmit the light to the lesion have become more flexible and reliable (Allison *et al.*, 2004c). Although modern fiber-optic systems and different types of endoscopes can target light more accurately to almost any part of the body, custom-sized and custom-shaped fibers are needed to achieve more homogenous illumination (Brown *et al.*, 2004; Allison *et al.*, 2006). Two other issues related to the use of light sources in PDT are: (i) the accurate calibration of any light source used, and (ii) monitoring of both light and drug delivery (drug and light dosimetry). Devices that could simultaneously monitor both light delivery and sensitizer fluorescence would greatly advance PDT as a more routine clinical treatment (Gudgin Dickson *et al.*, 2002).

(1.3) Photosensitizers

Thousands of natural and synthetic photoactive compounds have photosensitizing potential. They include degradation products of chlorophyll, polyacetylenes, thiophenes, quinones (cercosporin), anthraquinones (fagopyrin, hypericin), and 9-methoxypsoralen (Ebermann *et al.*, 1996). An ideal photosensitizer should be non-toxic, and should display local toxicity only after activation by illumination. The majority of the sensitizers used clinically belong to dyes, the porphyrin-chlorin platform, and furocoumarins (Fig. 2) (Allison *et al.*, 2004a; Meisel and Kocher, 2005).

The requirements of an optimal photosensitizer include photo-physical, chemical, and biological characteristics: (i)

highly selective tumor accumulation; (ii) low toxicity and fast elimination from the skin and epithelium; (iii) absorption peaks in the low-loss transmission window of biological tissues; (iv) optimum ratio of the fluorescence quantum yield to the inter-conversion quantum yield (The first parameter determines the photosensitizer diagnostic capabilities, and plays a key role in monitoring the photosensitizer accumulation in tissues and its elimination from them; the second parameter determines the photosensitizer ability to generate singlet oxygen.); (v) high quantum yield of singlet oxygen production *in vivo*; (vi) cost-effectiveness and commercial availability; (vii) high solubility in water, injection solutions, and blood substitutes; and (viii) storage and application light stability. The clinically relevant guidelines for the ideal photosensitizer have been summarized by Allison *et al.* (2004a).

Photofrin® (dihematoporphyrin ether), available for 30 years in its commercial form, and hematoporphyrin derivatives (HPDs) are referred to as first-generation sensitizers. Photofrin® is the most extensively studied and clinically used photosensitizer. Second-generation photosensitizers include 5-aminolevulinic acid (ALA), benzoporphyrin derivative (BPD), lutetium texaphyrin, temoporfin (mTHPC), tinethyletiopurpurin (SnET2), and talaporfin sodium (LS11). Foscan® (mTHPC), the most potent second-generation photosensitizer, has been reported to be 100 times more active than Photofrin® in animal studies (Allison *et al.*, 2004a). These photosensitizers have a greater capability to generate singlet oxygen; however, they can cause significant pain during therapy, and, because of their high activity, even dim light (60 Watt bulb) can lead to severe skin photosensitivity (Allison *et al.*, 2006). The third agent, ALA, is an intrinsic photosensitizer that is converted *in situ* to a photosensitizer, protoporphyrin IX. Topical ALA and its esters have been used to treat pre-cancer conditions, and basal and squamous cell carcinoma of the skin (Peng *et al.*, 1997; Brown *et al.*, 2004). An improvement of the tumor selectivity of photosensitizers is a major issue in PDT. Third-generation photosensitizers include currently available drugs that are modified by targeting with monoclonal antibodies or with non-antibody-based protein carriers and protein/receptor systems, and conjugation with a radioactive tag (Vrouenraets *et al.*, 2003; Sharman *et al.*, 2004; Allison *et al.*, 2006; Solban *et al.*, 2006). The cellular markers used for photodynamic targeting are mainly tumor surface markers, including growth factor receptors, low-density lipoprotein receptors, transferrin receptors, folic acid receptors, glucose transporters, integrin receptors, and insulin receptors. Only a few studies have been performed to target tumor endothelial markers (*e.g.*, Chen *et al.*, 2006). Large multi-institutional studies with appropriate dosimetric considerations and unbiased interpretation of results are nevertheless required, to verify the potential of targeted PDT (Allison *et al.*, 2004a). Currently, only four photosensitizers are commercially available: Photofrin®, ALA, Visudyne™ (BPD; Verteporfin), and Foscan®. The first three have been approved by the FDA, while all four are in use in Europe.

(1.4) Side-effects

The major side-effect after the use of intravenous photosensitizers is photosensitivity. Systemic administration of the sensitizer results in a period of residual skin photosensitivity, due to accumulation of the photosensitizer in the skin. This photosensitizer can be activated by daylight, causing first- or second-degree burns. Therefore, exposure to bright light or direct sunlight must be carefully avoided, to prevent sunburn, redness, and swelling, for a period ranging from several hours or weeks until the drug is eliminated. Photosensitivity reactions can occur in minutes, so it is important to take precautions to shield the skin and eyes from intense light exposure. Some photosensitizers may remain at significant concentrations in the skin for months, requiring a change in lifestyle. When repeated illuminations are necessary, such a prolonged photosensitivity may be of benefit. For most patients, however, a fast-acting and -eliminating photosensitizer would have an advantage, allowing patients to return to their normal routines. Systemic photosensitivity does not occur only in the case of topically applied ALA. Usually, PDT treatment by itself is not painful, but several hours after PDT, most patients suffer from severe pain. Pain medications should be given after or prior to the laser treatment. Patients treated by topically applied ALA report burning sensations during illumination. Photosensitizers have a tendency to accumulate in tumors, and the activating light is focused on the tumor. As a result, damage to healthy tissue is minimal; nevertheless, PDT can cause burns, swelling, pain, and scarring in nearby healthy tissues. Other side-effects of PDT are related to the area that is treated. They can include coughing, trouble swallowing, stomach pain, painful breathing, or shortness of breath; these side-effects are usually temporary. All other potential side-effects, such as allergic reaction, change of liver parameters, etc., occur infrequently and are specific for each photosensitizer and each patient (Vrouenraets *et al.*, 2003; Kübler, 2005).

(2) ANTICANCER THERAPY

Head and neck cancer is the term given to a variety of malignant tumors that develop in the oral cavity, the pharynx, the nasal cavity, and the larynx. Factors known to contribute to the risk of developing head and neck cancer include age, sunlight (for lip cancers), alcohol abuse, and smoking or other tobacco use. Most head and neck cancers are squamous cell carcinomas (SCCs); however, other tumor types may also be seen. Oral SCC is the most frequent malignant tumor of the oral cavity and the eighth most common cancer in the world, representing 2-4% of annually diagnosed cancers (Massano *et al.*, 2006). Despite numerous advances in surgery, chemotherapy, and radiation, the five-year survival rate has not improved significantly over the last 50 years. These conventional treatments cause many side-effects, including jaw pain, mouth sores, dysfunctional salivary glands, and difficulties in chewing, swallowing, and talking (Silverman, 1999). Oral SCCs develop generally from pre-malignant lesions of the oral mucosa. Erythroplakias and dysplastic leukoplakias are the most common pre-cancerous lesions, and about half of oral SCCs show signs of associated leukoplakia (van der Waal and Axéll, 2002). PDT-based therapy may have potential in different clinical presentations of head and neck cancer, including pre-malignant, primary, recurrent, and metastatic lesions.

(2.1) Photodynamic Therapy in Head and Neck Cancer

The advantage of PDT over conventional treatments is based on its minimal invasiveness and selective tumor destruction, with the preservation of healthy tissues. Some of the photosensitizers have the desirable property of concentrating in tumors (and certain other kinds of proliferating tissue) relative to the surrounding healthy tissue (Dougherty *et al.*, 1998). These features of PDT are important for head and neck squamous cell

Table 2. Photodynamic Therapy with Photofrin® and HPD/Photofrin® for Head and Neck Cancer[a]

Study	Location/Stage	Photosensitizer	Complete Response (%)
Keller *et al.* (1985)	Oral cavity, T1/T2	HPD/Photofrin®	100
Schuller *et al.* (1985)	Oral cavity/pharynx Localized recurrence	HPD/Photofrin®	100
Edge and Carruth (1988)	Oral cavity/pharynx Recurrent	HPD/Photofrin®	40
Zhao *et al.* (1989)	Lip, T1/T2	HPD/Photofrin®	100
Freche and De Corbiere (1990)	Larynx/T1	HPD/Photofrin®	78
Wenig *et al.* (1990)	Oral cavity/pharynx Local recurrence	Photofrin®	77
Gluckman (1991)	Oral cavity/larynx/T1	HPD/Photofrin®	87
Schweitzer (1990, 2001)	Oral cavity/T1	Photofrin®	80
Grant *et al.* (1993a)	Oral cavity/Tis/T1 (field cancerization)	Photofrin®	91
Feyh (1995, 1996)	Oral cavity/T1	Photosan	85
	Larynx	Photosan	92
Kulapaditharom and Boonkit (1996, 1999, 2000)	Oral cavity/pharynx Tis/T1/T2	HPD/Photofrin®	73
Biel (1996b, 1998, 2002)	Larynx/T1	Photofrin®	89
	Oral cavity/pharynx T1/T2	Photofrin®	92
	Various/advanced	Photofrin®	50
	Various/intra-operative		65

[a] Adapted from Allison *et al.* (2005) and Biel (2002, 2006).

Table 3. Photodynamic Therapy with Foscan® for Head and Neck Cancer[a]

Study	Location/Stage	Photosensitizer	Complete Response (%)
Grosjean *et al.* (1996)	Oral cavity/Tis	Foscan®	100
Fan *et al.* (1997)	Oral cavity/Tis/T1/T2	Foscan®	80
	T3/T4		57
	Diffuse		64
Kübler *et al.* (2001)	Lip/ Tis/T1/T2	Foscan®	92
Dilkes *et al.* (1996, 2003)	Larynx/T1/T2	Foscan®	25
	Oral cavity/pharynx/T1/T2		93
	Oral cavity/pharynx/T3		40
	Palliative		29
	Oral cavity/pharynx/neck Adjuvant therapy		43
Cooper *et al.* (2003)	Oral cavity/oropharynx/T1	Foscan®	95
	T2		57
Hopper *et al.* (2004a)	Oral cavity/pharynx/lip/T1	Foscan®	93
	T2		58
D'Cruz *et al.* (2004)	Oral cavity/pharynx	Foscan®	
	All recurrence		16
	Limited recurrence		30
Lou *et al.* (2004)	Various/recurrent	Foscan®	20
	Neck/recurrent		0

[a] Adapted from Allison *et al.* (2005) and Biel (2002, 2006).

carcinoma (SCC), in which excessive tissue loss causes considerable functional problems. In addition, PDT may be applied in combination with conventional treatments (Biel, 2002). Clinical applications and outcomes of PDT in the treatment of head and neck SCC have been reviewed by Biel (2002, 2006) and Allison *et al.* (2005). The FDA has not approved PDT for the treatment of head and neck SCC; in clinical trials, all patients are treated according to specific protocols in accordance with the FDA and the local Institutional Review Board (IRB) approval. In the EU, Foscan®-based PDT has been approved for the treatment of early head and neck cancers, and for palliative treatment of head and neck cancer (Biel, 2006). The clinical data reported for Photofrin®-, HPD-, and Foscan®-based PDT are summarized in Tables 2 and 3. At this time, data are available for over 1300 patients who received PDT by Photofrin®, HPD, Foscan®, or ALA for the treatment of head and neck cancer (Fig. 3). Patients had different types of cancerous lesions, including primary, recurrent, and metastatic lesions. The prevalent histology was SCC, but others included mucosal melanoma, Kaposi's sarcoma, adenocarcinoma, metastatic breast carcinoma, and adenoid cystic carcinoma (Biel, 2002).

(2.2) Photofrin® and Derivatives

Photofrin® is the most extensively studied and clinically used photosensitizer. More than 10,000 patients with different types of cancer have been treated with this drug. Photofrin® is a registered trademark of Axcan Pharma PDT Inc., used under license by Axcan Pharma Ltd. (Ireland). Photofrin® is injected intravenously, usually at 2 mg/kg in an outpatient setting, and after 48 hrs, the tumor is illuminated at 630 nm. At this wavelength, light penetrates 0.5 to 1.0 cm into the tissue; Photofrin® has limited application in the treatment of large solid tumors. The clinical results are generally excellent. The drug seems reliable, easy to activate, pain-free, and non-toxic; however, it is not highly selective at 2 mg/kg. The major side-effect of Photofrin® is significant prolonged skin photosensitivity observed up to 6 weeks after treatment. These extensive normal tissue reactions can be reduced by photo-bleaching, the treatment that utilizes a lower drug dose (Biel, 2002; Allison *et al.*, 2004a). It has been proposed that since Photofrin® accumulates usually a little bit more in the tumor than in surrounding healthy tissues, it may be possible to decrease the dosage and still obtain a clinically relevant photodynamic reaction in the tumor. Allison *et al.* (2004a) reported that Photofrin® at 1.2 mg/kg works very well in malignant lesions of the oral cavity and pharynx, without fibrotic changes or significant morbidity.

Patients with early-stage cancers and early recurrences in

the oral cavity and larynx (Tis/T1/T2) usually respond very well to Photofrin®-based PDT (Table 2). The largest groups of head-and-neck SCC patients treated with Photofrin® have been examined by Biel (1996a,b, 1998, 2002). A major finding was a highly successful treatment of early 'true' larynx cancer. Even in patients who failed an initial therapy (usually radiation), a complete response was observed in ~ 90%. PDT should be considered as an option for the treatment of primary and recurrent Tis, T1, and T2 SCC of the larynx (Biel, 2006). Photofrin®-based PDT is also effective in the treatment of primary and recurrent carcinomas, Tis and T1, of the oral cavity (Table 2). Biel (1996b, 2002) reported the first human clinical trials with long-term follow-up, using Photofrin® as an intra-operative adjuvant therapy for recurrent head and neck cancer. The post-operative course was uncomplicated, and the treatment improved the cure rates significantly (Biel, 2002, 2006). Photofrin® has also been used in patients with advanced tumors that were untreatable or refractory to conventional therapies. Almost all patients had a partial response, but the tumor grew back after therapy ceased (reviewed in Biel, 2002). Recently, Photofrin® has been used for the treatment of maxillary gingival SCC, preventing maxillectomy and radiation therapy. The patient returned for evaluation at 18 and 25 months after PDT, and had no clinical recurrence (Mang *et al.*, 2006).

(2.3) Foscan® (Temoporfin; mTHPC)

Foscan® [5,10,15,20-meta-tetra(hydroxyphenyl)chlorin, Temoporfin, mTHPC], a potent second-generation photosensitizer, is commercially available from Biolitec Pharma Ltd. (Dublin, Ireland). In October, 2001, Foscan® was approved in the European Union, Norway, and Iceland as a local therapy for the palliative treatment of patients with advanced head and neck cancer, for whom previous therapies have failed, and who are unsuitable for radiotherapy, surgery, or systemic chemotherapy. The aims of Foscan®-based PDT include preservation of organ function, local tumor destruction, relief of symptoms, and avoidance of disease-related complications. There is a delay of 4 days between the injection of Foscan® into the bloodstream, usually at 0.15 mg/kg, and activation with laser light at 652 nm. This allows for the accumulation of Foscan® in cancer cells. The intravenous administration of Foscan® is associated with pain. In head and neck tumors, where Foscan® is commonly used, large blood vessels cover these regions, and deep penetration leading to vascular damage could be devastating. As with other photosensitizing agents, administration of Foscan® results in light sensitivity for a period of approx. 15 days, and appropriate light exposure precautions should be followed during this period. The clinically relevant guidelines and potential shortcomings of Foscan®-based PDT have been summarized by Allison *et al.* (2004a).

The use of Foscan®-based PDT by individual investigators demonstrated its efficiency for early oral and pharyngeal cancers (Table 3). Large multicenter Phase II trials have been performed recently to evaluate the efficacy of Foscan® PDT in the treatment of primary oropharyngeal cancers, and recurrent and second primary oral carcinomas. Hopper *et al.* (2004a) reported excellent results for early oral SCC. The trial involved 114 patients with T1-T2 oropharyngeal cancers. The patients received Foscan® intravenously at 0.15 mg/kg and had three light exposures at 652 nm. Overall, 93% and 58% of complete response was shown for T1 and T2 lesions, respectively. Most patients had floor-of-the-mouth, lip, and anterior tongue lesions. All patients sustained an excellent functional status after PDT, and none of them required airway management. D'Cruz *et al.* (2004) reported data on 128 patients with incurable or recurrent disease. Fifteen patients had multiple lesions. About 16% of patients achieved a complete response. Thus, it appears that this group of patients, who had already had extensive surgery and radiation, could still benefit from 'salvage' PDT. Biel (2006) evaluated Foscan® PDT in 96 patients with recurrent or second carcinomas in the oral cavity, and reported a 50% complete response rate, confirmed histologically, and a 79% survival rate. Foscan® may be effective in the treatment of lip cancer, especially due to better cosmetic and functional results compared with those achievable by surgery and radiation (Kübler *et al.*, 2001). Foscan®-based PDT is a cost-effective treatment option for patients suffering from early oral SCC (Hopper *et al.*, 2004b) and advanced head and neck cancer (Kübler *et al.*, 2005). For patients who have had very limited treatment possibilities until now, Foscan®-based PDT offers a chance for reduction of the tumor, remission, and a prolonged life expectancy (Hopper *et al.*, 2004b). Despite these promising results, Foscan® has not received FDA approval for head and neck SCC treatment in the US.

(2.4) 5-Aminolevulinic acid (ALA)

A pro-drug, 5-aminolevulinic acid (ALA), serves as a precursor of the photosensitizer, protoporphyrin IX (PpIX), in the heme biosynthetic pathway (Fukuda *et al.*, 2005). Exogenous ALA administration inhibits the first step of porphyrin synthesis, resulting in the accumulation of PpIX in the tissue. Due to the limited depth of topical ALA, and the limited light penetration at 635 nm, the use of ALA is restricted to superficial lesions (1-2 mm); the treatment of early-stage head and neck cancer with ALA-PDT has been ineffective (Grant *et al.*, 1993b; Fan *et al.*, 1996; Sieron *et al.*, 2001). ALA is rapidly cleared from the tissues and the body within 48 hrs, and skin photosensitivity lasts less than 24 hrs.

Oral leukoplakia (OL) and oral verrucous hyperplasia (OVH) are two common pre-malignant lesions that may transform into squamous cell carcinoma or verrucous carcinoma (VC). The presence of dysplasia in leukoplakia lesions increases the occurrence of malignancy by over 30% (Sieron *et al.*, 2003). PDT with orally or topically administered ALA has been used for the treatment of pre-malignant and malignant lesions in the oral cavity (Table 4). PDT of the oral mucosa causes superficial necrosis, leaving little scarring and no cumulative toxicity (Kübler, 2005). Two ALA preparations, Metvix® (PhotoCure ASA, Oslo, Norway) and Levulan® Kerastic™ (DUSA Pharmaceuticals, Wilmington, MA, USA), have been approved by the European Agency for the Evaluation of Medicinal Products (EMEA) and the FDA, respectively, for the treatment of non-hyperkeratotic actinic keratoses of the face and scalp (Gold and Goldman, 2004).

The clinical data reported for ALA-based PDT for the treatment of oral leukoplakia are summarized in Table 4. Fan *et al.* (1996) treated 12 patients with oral dysplastic lesions using orally administered ALA. All 12 patients showed regression of the lesions to normal or less dysplastic. Kübler *et al.* (1998) treated 12 patients who had been suffering from leukoplakia of the oral mucosa for several years. ALA (20% cream) was applied to the leukoplakia lesion of the oral mucosa for 2 hrs. Five patients showed complete response, four patients showed a partial response, and in three patients treatment was

Table 4. Photodynamic Therapy of Oral Pre-malignant and Malignant Lesions with 5-Aminolevulinic Acid

Study	Site Lesion	Photosensitizer Light (nm)/Laser	Patients, n (Response, n)
Kübler *et al.* (1996)	Oral mucosa	ALA (20% cream)	6 (2 CR)
	Leukoplakia	630 APL	(3 PR)
			(1 none)
Fan *et al.* (1996)	Mouth	ALA (oral)	18 (14 CR)
	Pre-malignant/malignant lesions	628 (laser light)	12 (12 CR)
	-Dysplasia, -SCC		6 (2 CR)
Kübler *et al.* (1998)	Oral mucosa	ALA (20% cream)	12 (5 CR)
	Leukoplakia	630 APL	(4 PR)[a]
			(3 none)
Sieron *et al.* (2001)	Oral leukoplakia	ALA (10% cream)	5 (4 CR)
Sieron *et al.* (2003)	Buccal, gingival	ALA (10% cream)	12 (10 CR)[b]
	mandibular mucosa	635 APL	(2 none)
	Leukoplakia		
Tsai *et al.* (2004)	Oral mucosa	ALA (20% gel)	24 (3 CR)
	Leukoplakia	635 LED	(9 PR)
			(12 none)
Chen *et al.* (2004)	Mouth angles	ALA (20% gel)	5 (5 CR)
	Labial and buccal mucosa	635 LED	
	Verrucous hyperplasia		
HM Chen *et al.* (2005a)	Mouth angle, buccal mucosa	ALA (20% gel)	1 (1 CR)
	Extra-oral verrucous carcinoma	635 LED	
HM Chen *et al.* (2005b)	Oral verrucous hyperplasia	ALA (20% gel)	8 (8 CR)
	Oral leukoplakia lesions	635 LED	24 (8 CR)
			(16 PR)
Franco (2006)	Recalcitrant leukoplakia	ALA (20% cream)	12 (9 CR)
		585 (pulsed dye laser)	

[a] One patient with partial response was re-treated, after which the lesion disappeared.
[b] One recurrence; CR, complete response; PR, partial response; APL, argon-pumped laser; LED, light-emitting diode.

unsuccessful. Using 10% ALA cream, Sieron *et al.* (2003) treated 12 patients with lesions that affected a variety of intra-oral sites. Irradiation was performed in several (6-8) sessions. A complete response was obtained in 10 patients, with one recurrence during 6 months. Chen *et al.* (2005b) treated eight patients with OVH and 24 patients with OL using the topical ALA-PDT (20% gel). A complete regression of OVH lesions was obtained after fewer than 6 treatments once a week, while all OL lesions had at least a partial response after 8 treatments twice a week. These results indicate that topical ALA-PDT is an effective treatment modality for cutaneous and mucosal pre-malignant lesions, including OVH and OL lesions. The observed variations in the treatment results could be due to the different ALA preparations, the number of ALA applications (single or multiple), the incubation period (1.5-5 hrs), the light source (laser or LED), the illumination protocol (continuous or fractionated), and the number of treatments (single or multiple). These may be attributed to its low invasiveness, good tolerance, excellent cosmetic effect, ability to treat multifocal lesions, and repeated use without the risk of toxicity. ALA-based PDT may be an alternative to conventional treatments for superficial lesions such as epithelial dysplasias, but is not sufficient for deep tumors. Further clinical studies are required to evaluate the effectiveness of PDT with ALA in the treatment of oral leukoplakia.

(3) PHOTODYNAMIC ANTIMICROBIAL CHEMOTHERAPY OF DENTAL AND MUCOSAL INFECTIONS

It has been known since the beginning of the last century that micro-organisms can be killed by the combination of dyes and light, but the interest in antimicrobial PDT was hampered by the introduction of antibiotics. In recent years, the emergence of antibiotic-resistant strains, such as methicillin-resistant *Staphylococcus aureus* and vancomycin-resistant *Enterococcus faecalis,* stimulated a search for alternative treatments. PACT has the potential to be such an alternative, especially for the treatment of localized infections of the skin and the oral cavity. Micro-organisms that are killed by PACT include bacteria, fungi, viruses, and protozoa. The development of resistance to PACT appears to be unlikely, since, in microbial cells, singlet oxygen and free radicals interact with several cell structures and different metabolic pathways. PACT is equally effective against antibiotic-resistant and antibiotic-susceptible bacteria, and repeated photosensitization has not induced the selection of resistant strains (Wainwright and Crossley, 2004). Anti-oxidant enzymes, such as superoxide dismutase and catalase, protect against some oxygen radicals, but not against singlet oxygen. The photosensitizer can be delivered to infected areas by topical application, instillation, interstitial injection, or aerosol delivery. Several publications have summarized the photobiology of PACT, and its potential for the treatment of localized infections (Hamblin and Hasan, 2004; Wainwright and Crossley, 2004; O'Riordan *et al.*, 2005; Meisel and Kocher, 2005; Smith, 2005; Kömerik and MacRobert, 2006). A few studies have evaluated the use of PACT in animal models or in clinical trials, mainly for viral lesions, acne, gastric infection by *Helicobacter pylori*, and brain abscesses (reviewed by Hamblin and Hasan, 2004; O'Riordan *et al.*, 2005). The *in vitro* effect of PACT has been investigated primarily against micro-organisms growing in liquid (planktonic) cultures. Here, we review the use of PACT for the treatment of oral biofilms *in vitro*, and *in vivo* studies on the treatment of oral infections.

(3.1) Photosensitizers

Photosensitizers used in PACT include: (i) phenothiazine dyes [Methylene Blue (MB) and Toluidine Blue O (TBO; tolonium chloride)]; (ii) phthalocyanines [aluminum disulphonated phthalocyanine and cationic Zn(II)-phthalocyanine]; (iii) chlorines [chlorin e6, Sn(IV)chlorin e6, chlorin e6-2.5 N-methyl-d-glucamine (BLC1010)], and polylysine and

polyethyleneimine conjugates of chlorin e6; (iv) porphyrins (hematoporphyrin HCl, Photofrin®, and ALA); (v) xanthenes (erythrosin); and (vi) monoterpene (azulene) (Wainwright, 1998). The photosensitivity of bacteria appears to be related to the charge of the sensitizer. In general, neutral or anionic photosensitizers bind efficiently to and inactivate Gram-positive bacteria, while they bind to some extent to the outer membrane of Gram-negative bacteria, but do not inactivate them after illumination. A relatively porous layer of peptidoglycan and lipoteichoic acid outside the cytoplasmic membrane of Gram-positive species allows the photosensitizer to diffuse into sensitive sites. The outer membrane of Gram-negative bacteria acts as a physical and functional barrier between the cell and its environment. The affinity of negatively charged photosensitizers for Gram-negative bacteria may be enhanced by linking the sensitizer to a cationic molecule (*e.g.*, poly-L-lysine-chlorin e6), by the use of membrane-active agents (*e.g.*, treatment with Tris-EDTA), or by conjugating the sensitizer with a monoclonal antibody that binds to cell-surface-specific antigens (Wainwright, 1998; Rovaldi *et al.*, 2000; Hamblin and Hasan, 2004; Kömerik and MacRobert, 2006).

Table 5. Photodynamic Antimicrobial Chemotherapy Studies on Plaque Biofilms *in vitro*

Study	Photosensitizer*	Light (nm)/Laser	Micro-organisms
Dobson and Wilson (1992)	TBO, MB, AlS2Pc HP-HCl	633 He/Ne	*S.s., P.g., F.n., A.a.*
Wilson *et al.* (1996)	AlS2Pc	660 LED	*S.s.*
Haas *et al.* (1997)	TBO	905 LED	*A.a., P.g., P.i.*
Wood *et al.* (1999)	ZnPc	white light	mixed strains
O'Neill *et al.* (2002)	TBO	633 He/Ne	mixed strains
Seal *et al.* (2002)	TBO	633 He/Ne	*S.i.*
Soukos *et al.* (2003)	pL-Ce6	red light	*A.n.*
Lee *et al.* (2004)	ALA	630 LED	*P.a.*
Zanin *et al.* (2005)	TBO	633 He/Ne; 639 LED	*S.m.*
Metcalf *et al.* (2006)	Erythrosin	white light	*S.m.*
Hope and Wilson (2006)	SnCe6	488 Ar; 543 He/Ne	*S.p.*
Wood *et al.* (2006)	Erythrosin MB, Photofrin®	white light	*S.m.*
Williams *et al.* (2006)	TBO	633 LED	*S.i.*
Zanin *et al.* (2006)	TBO	639 LED	*S.m., S.s., S.sob.*
Soukos *et al.* (2006)	MB	665 LED	*E.f.*
Donnelly *et al.* (2007)	TBO	635 Paterson lamp	*C. albicans*
Garcez *et al.* (2007)	PEI-Ce6	660 LED	*P.m., P.a.*

* AlS2Pc, aluminum disulphonated phthalocyanine; HP-HCl, hematoporphyrin HCl; MB, methylene blue; PEI-Ce6, polyethyleneimine-chlorin e6 conjugate; pL-cE6, polylysine-chlorin e6 conjugate; SnCe6, Sn(IV)chlorin e6; TBO, toluidine blue O; ZnPc, Zn(II)-phthalocyanine; LED, light-emitting diode; He/Ne, helium/neon laser; *A.a., Actinomyces actinomycetemcomitans; A.n., Actinomyces naeslundii; E.f., Enterococcus faecalis; F.n., Fusobacterium nucleatum; P.a., Pseudomonas aeruginosa; P.i., Prevotella intermedia; P.g., Porphyromonas gingivalis; P.m., Proteus mirabilis; P.mic., Peptostreptococcus micros; S.i., Streptococcus intermedius; S.m., Streptococcus mutans; S.p., Streptococcus pyogenes; S.s., Streptococcus sanguinis; S.sob., Streptococcus sobrinus.*

(3.2) Effects of PACT on Oral Biofilms

The oral cavity is colonized by complex, relatively specific, and highly interrelated micro-organisms, including aerobic and anaerobic Gram-positive and Gram-negative bacteria, fungi, mycoplasma, protozoa, and viruses. Dental plaque can be defined as the diverse community of micro-organisms found on the tooth surface as a biofilm, embedded in an extracellular matrix of polymers (EMP) of host and microbial origin. In the biofilm, bacteria exhibit increased resistance to antibiotics, environmental stresses, and host immune defense mechanisms. Two of the most common bacterial diseases that afflict humans are dental caries and periodontal diseases. Both result originally from a build-up of plaque biofilms on the teeth and soft tissues of the mouth. Mechanical removal of plaque, good oral hygiene, and antimicrobial agents are the most common treatments for periodontitis. Nevertheless, the limited access of topical agents to the plaque and the development of antibiotic-resistance create the necessity for alternative strategies to control plaque and to treat gingivitis and periodontal diseases.

The antimicrobial activity of photosensitizers is mediated by singlet oxygen, which, because of its high chemical reactivity, has a direct effect on extracellular molecules. Thus, the polysaccharides present in EMP of a bacterial biofilm are also susceptible to photodamage. Such dual activity, not exhibited by antibiotics, represents a significant advantage of PACT. Breaking down biofilms may inhibit plasmid exchange involved in the transfer of antibiotic resistance, and disrupt colonization.

The activity of PACT against homogenous and mixed Gram-positive/Gram-negative oral biofilms has been reported for a range of photosensitizers (Table 5). Electron microscope evidence for the destruction of biofilm structure has been observed for dental-type biofilms treated with Zn(II)-phthalocyanine (Wood *et al.*, 1999). Biofilms of the oral pathogen *Actinomyces viscosus* have been exposed to red light in the presence of poly-L-lysine-chlorin(e6) conjugate (pL-Ce6). Confocal microscopy revealed that a photochemical wave increased the penetration of the pL-Ce6 conjugate by 50% and caused killing of 99% of biofilm bacteria (Soukos *et al.*, 2003). Over 97% of oral bacteria were killed in multi-species biofilms irradiated with light from a helium/neon laser in the presence of TBO (O'Neill *et al.*, 2002). Biofilms of *Streptococcus mutans* were subjected to PACT with erythrosin, MB, and Photofrin® as photosensitizers, and white-light irradiation (500-650 nm). Erythrosin was significantly more effective than either MB or Photofrin®. With all three photosensitizers, PACT was increasingly more effective as the biofilm age increased, suggesting that "young" biofilms are less susceptible than "older" biofilms (Wood *et al.*, 2006). In contrast, Zanin *et al.* (2005), using TBO as a photosensitizer, reported that younger biofilms of *S. mutans* are more sensitive to PACT. The reasons for this difference are unclear, but may be due to the properties of the photosensitizers used and/or the differences in extracellular matrix composition.

In addition to treatment for periodontitis, the use of PACT

Table 6. *In vivo* Studies on Photodynamic Antimicrobial Chemotherapy

Study	Photosensitizer*	Light (nm)/Laser	Micro-organisms
Haas *et al.* (2000)	TBO	906 LED	peri-implantitis, 17 patients
Dortbudak *et al.* (2001)	TBO	690 LED	*A.a., P.g., P.i.,* 15 patients
Teichert *et al.* (2002)	MB	664 LED	*C. albicans*, mice
Kömerik *et al.* (2003)	TBO	630 LED	*P.g.,* rats
Shibli *et al.* (2003)	TBO	685 LED	*P.i., P.n., S.b., Fusobac.* sp., dogs
Hayek *et al.* (2005)	Azulene	660 LED	*S.b., Fusobac.* sp., *Prevotella* sp., dogs
Sigusch *et al.* (2005)	Ce6, BLC1010	662 LED	*P.g., F.n.*, dogs
Shibli *et al.* (2006)	TBO	830 LED	peri-implantitis, dogs

* Ce6, chlorin e6; MB, methylene blue; TBO, toluidine blue O; LED, light-emitting diode; *A.a., Actinomyces actinomycetemcomitans; F.n., Fusobacterium nucleatum; P.i., Prevotella intermedia; P.g., Porphyromonas gingivalis; P.n., Prevotella nigrescens; S.b., Streptococcus beta-haemolyticus.*

for peri-implantitis and endodontic treatment has also come into focus. Biofilms of *Streptococcus intermedius* prepared in artificial root canals and extracted human teeth were subjected to PACT with TBO and a laser diode device (633 nm) equipped with an endotip that allowed light to be transmitted down to the apex of the tooth. *S. intermedius* was present in numbers similar to those found in heavily infected root canals. Photoactivated disinfection significantly reduced the number of bacteria in both types of root canals (Williams *et al.*, 2006). Seal *et al.* (2002) reported partial inactivation of *S. intermedius* biofilms in root canals of extracted teeth, using TBO and a helium/neon laser (633 nm). MB in combination with red light irradiation (665 nm) was able to eliminate 97% of *E. faecalis* biofilm bacteria in root canals of extracted teeth (Soukos *et al.*, 2006). Biofilms of *Proteus mirabilis* and *Pseudomonas aeruginosa*, prepared in extracted human teeth, were treated with a conjugate of polyethylenimine with chlorin(e6), followed by illumination with a diode laser (660 nm). Bioluminescence imaging was used to quantify the bacterial burden. PACT alone reduced the bioluminescence by 95%, while the combination of standard endodontic treatment with PACT reduced bioluminescence by > 98% (Garcez *et al.*, 2007). Electron microscopy revealed complete eradication of bacteria in uniform biofilms of *Aggregatibacter actinomycetemcomitans*, *Porphyromonas gingivalis*, or *Prevotella intermedia*, prepared on different implant surfaces treated with TBO and irradiated with a diode soft laser (905 nm) (Haas *et al.*, 1997).

Oropharyngeal candidiasis, caused by *Candida albicans* and related *Candida* sp., is a widespread opportunistic infection in HIV-infected individuals and in patients taking immunosuppressive drugs. *Candida*-associated denture stomatitis is a common recurrent disease in denture wearers. The ability of *C. albicans* to form biofilms on epithelial surfaces and prosthetic devices contributes to the failure of antifungal therapy and to recurrent infections (Douglas, 2003). The increasing resistance of *C. albicans* to antifungal agents has stimulated an interest in the new treatments. Similarly to other yeasts, *C. albicans* is more difficult to kill by PACT than are Gram-positive bacteria. This is attributed to the presence of a nuclear membrane and the larger cell size (Demidova and Hamblin, 2005). Recently, Donnelly *et al.* (2007) reported on muco-adhesive patches containing TBO as a potential delivery system in oral candidiasis. The authors also investigated the effect of PACT on *C. albicans* biofilms using TBO and illumination with a Paterson lamp (635 nm). With biofilms, higher concentrations of TBO and longer incubation times were required to achieve a total 'kill' than for planktonic cells. The reduced susceptibility of biofilm-grown *C. albicans* to PACT was surprising, since it has been shown previously that bacteria growing both planktonically and in biofilms are equally susceptible to PACT (Lee *et al.*, 2004). The different susceptibility observed here may be explained by the structural differences between bacterial and yeast biofilms, or the inability of the light to penetrate thick candidal biofilms.

(3.3) *In vivo* Studies of the Efficiency of PACT in Dental and Mucosal Infections

There have been only a few studies investigating the efficacy of PACT in clinical trials and in animal models of oral infections (Table 6). Peri-implantitis is a multifactorial process involving bacterial contamination of the implant surface and the formation of biofilms. Bacterial plaque on implants leads to inflammatory changes in the adjacent soft tissues. An application of TBO on implant surfaces in 15 patients with peri-implantitis, followed by illumination with a diode soft laser (690 nm), significantly reduced the numbers of *A. actinomycetemcomitans*, *P. gingivalis*, and *P. intermedia* (Dortbudak *et al.*, 2001). Under similar conditions, PACT was also effective in decreasing the extent of inflammation in 17 patients with peri-implantitis (Haas *et al.*, 2000).

Kömerik *et al.* (2003) investigated whether *P. gingivalis* can be killed by TBO-mediated PACT in an animal model without damage to the adjacent periodontal tissue, and whether such treatment has any effect on bone destruction associated with periodontitis. When the gingival crevices in maxillary molars of rats were inoculated with *P. gingivalis* and exposed to light from a diode laser (630 nm) in the presence of TBO (1 mg/mL), no viable bacteria were detected. In addition, the photosensitization procedure significantly reduced alveolar bone loss. The clinical use of TBO-mediated PACT appears unlikely, because it colorizes teeth. Sigusch *et al.* (2005) established a clinical infection model using beagle dogs. The animals were infected with *P. gingivalis* and *Fusobacterium nucleatum* in all subgingival areas. PACT was conducted with two sensitizers, chlorin e6 and a novel water-soluble chlorin e6 derivative, BLC1010, followed by illumination with a diode laser (662 nm). The treatment caused a significant reduction in redness and bleeding on probing; *P. gingivalis* was much more sensitive to PACT than was *F. nucleatum*.

Shibli *et al.* (2003) investigated the effects of TBO-mediated PACT with a GaAlAs diode laser (685 nm) on ligature-induced peri-implantitis in dogs. PACT reduced bacterial counts of *Prevotella* sp., *Fusobacterium* sp., and *Streptococcus beta-haemolyticus.* The association of PACT with guided bone regeneration (GBR) in the treatment of peri-implantitis in dogs allows for better re-osseointegration than with GBR alone (Shibli

et al., 2006). Hayek *et al.* (2005) used a ligature-induced peri-implantitis model in dogs with natural bacterial plaque. The effect of PACT was compared with that of conventional peri-implantitis treatment, consisting of a mucoperiosteal flap and irrigation with chlorhexidine. A paste-based azulene sensitizer was placed into the peri-implant defect, followed by irradiation with a GaAlAs diode laser (660 nm). Both treatments significantly reduced the *Prevotella* sp., *Fusobacterium* sp., and *S. beta-haemolyticus* counts, with no significant differences between the two treatments. The use of azulene delivered in a paste does not stain the implant surface and/or surrounding tissues.

In immunosuppressed mice, topical application of MB at 450 and 500 μg/mL, followed by illumination with a diode laser (664 nm), totally eradicated *C. albicans* from pseudomembranous candidiasis lesions on the dorsum of the tongue (Teichert *et al.* 2002). Further clinical studies are required to evaluate the use of PACT in the treatment of oral infections.

(4) PERSPECTIVES AND FUTURE DIRECTIONS

The studies we have cited in this review indicate that PDT for the treatment of oral cancer and oral epithelial dysplastic lesions, as well as PACT for the treatment of oral infections, may have a significant potential for clinical applications. In the past few years, major progress has been made in assessment of the use of PDT as: (i) the treatment for early head and neck carcinomas; (ii) the palliative treatment for refractory head and neck cancer; (iii) an intra-operative adjuvant therapy, for recurrent head and neck cancer; and (iv) ALA-based PDT for the treatment of oral pre-malignant lesions. In general, however, PDT remains on the periphery of the treatment options for head and neck cancer, and is considered as a competitive rather than a complementary therapy. Thus far, the lack of accurate dosimetry and appropriate illumination devices, coupled with poorly defined treatment parameters, has diminished the success of PDT. The development of new, more tumor-specific photosensitizers and light delivery systems, and well-designed, randomized, and standardized controlled trials should improve the efficacy of PDT and accelerate the FDA's approval of its use for the treatment of head and neck cancers.

Successful PDT is limited by problems in dosimetry and sub-optimal photosensitizers. Until now, the improvement of currently available photosensitizers and the development of new photosensitizers for the treatment of cancer have emphasized their chemical properties, rather than biological and clinical characteristics. Studies have been focused predominantly on better optical properties of photosensitizers, while problems that should be addressed are associated with a sustained skin photosensitivity, low selectivity, and inconvenient drug-to-light intervals.

A moderate enhancement of photosensitizer accumulation in tumor tissues provides a first level of selectivity, while further selectivity may be provided by the homogenous illumination of the target area with a custom-size fiber optic. Developing such devices would be a much-needed future direction for PDT. The LED devices that can be shaped into numerous forms and sizes, and are cost-effective, may replace laser light sources and their fiber optics. Recently, Allison *et al.* (2006) have discussed the more far-reaching approaches, such as (i) a metronomic PDT that uses a continuous delivery of photosensitizer and light at low rates for extended periods of time, (ii) the concept of implantable light sources, and (iii) the attachment of bioluminescent material to photosensitizers.

Although the photo-antibacterial properties of, for example, methylene blue and toluidine blue have been known for a long time, the introduction of antibiotics in the 20th Century has hindered the progress of PACT. The increasing drug-resistance to all classes of antibiotics will necessitate the development of alternative treatments. Pre-clinical work has shown that photosensitizers are more toxic against microbial species than against mammalian cells, and that the illumination-based toxicity occurs much earlier in prokaryotic than in eukaryotic cells. PACT appears to be most efficient for treatment of localized and superficial infections. Thus, infections in the oral cavity—such as mucosal and endodontic infections, periodontal diseases, caries, and peri-implantitis—are potential targets. PACT will not replace antimicrobial chemotherapy, but the photodynamic approach may improve the treatment of oral infections, accelerating and lowering the cost of the treatment. Development of new photosensitizers, more efficient light-delivery systems, and further animal studies are required to establish the optimum treatment parameters before investigators can proceed to clinical trials and eventual clinical use.

(4.1) Recent Clinical Advances in PDT

Although the therapeutic potential of light-based treatments has been recognized for some time, the expansion of PDT has occurred only recently, due to its promising results and clinical simplicity. The fact that rapid cytotoxic and vasculotoxic reactions result in visible tumor destruction and sparing of normal tissue makes PDT appealing to both patients and clinicians. While PDT is currently applied mostly in oncological therapy, in the future it will most likely be applied to other areas. Clinical PDT is continuing to grow because of the relatively recent availability of portable and dependable light sources. Since no malignancy has been found to be genetically resistant to PDT, many histologically different tumors have undergone PDT. Clinical trials are under way to evaluate the use of PDT for cancers of the brain, breast, skin, prostate, cervix, pancreas, peritoneal cavity, and lymphatic system (Allison *et al.*, 2006). PDT offers high tumor control and low morbidity for vocal cord lesions (Hopper, 2000) and esophageal tumors (Overholt *et al.*, 2005). PDT could offer organ-sparing treatment worldwide with very little investment in infrastructure, and could be highly successful under relatively primitive conditions (D'Cruz *et al.*, 2004; Bagnato *et al.*, 2005). A new device based on LED technology (Light Sciences Corporation, Issaquah, WA, USA) allows for the production of light inside the target tissue. This new technology could expand the use of PDT for the treatment of moderate- and large-volume refractory tumors (Chen *et al.*, 2002).

PDT has also been used in the treatment of malignancies other than head and neck SCC. Gynecologic tumors are often localized in areas that are easily accessible to the activating light, and PDT may be an effective treatment in tumors involving the vagina, vulva, and cervix. PDT works well as a palliative treatment for breast cancers recurring in the chest wall (Allison *et al.*, 2004b; Cuenca *et al.*, 2004). Photofrin®-based PDT has been approved by the FDA for a few early- and late-stage lung tumors. The cancer must be located in the airways and be reachable by a bronchoscope (Allison *et al.*, 2004c). The treatment has been highly effective and minimally morbid compared with surgical interventions (Ost, 2000). The use of PDT has also been investigated for pleura-based tumors. The preliminary results have shown that PDT may offer

improved local control for the disease that has spread to the pleura from either mesothelioma or non-small-cell cancer (Friedberg *et al.*, 2003, 2004). The ability to palliate obstructing esophageal tumors was one of the first indications for PDT. In 1995, the FDA approved Photofrin®-based PDT for the treatment of advanced and obstructing esophageal cancer (Allison *et al.*, 2004c). In 2003, the FDA approved Photofrin® for the treatment of precancerous lesions in patients with Barrett's esophagus (a condition that can lead to esophageal cancer) (Wolfsen, 2000). One of the first successful reports of Photofrin®-mediated PDT was on bladder cancer. Based on strong clinical data, the Canadian government, in 1993, approved PDT for the treatment of recurrent superficial papillary bladder cancer (Allison *et al.*, 2004c). Excellent results have also been obtained with ALA-based treatment, which may reduce the risk of bladder fibrosis due to reduced tissue penetration (Koenig *et al.*, 1999). The skin has been an obvious choice for PDT. Almost all types of histology—including squamous cell, basal cell, melanoma, Kaposi's sarcoma, metastasic lesions, and lymphoma—respond to Photofrin®-based PDT (Allison *et al.*, 2004c, 2006). Despite these excellent results, the treatment has not obtained FDA approval for skin cancers. Photofrin®-based PDT has shown a successful outcome in several non-malignant cutaneous conditions. FDA approval has been obtained for ALA-based therapy of actinic keratosis (Itoh *et al.*, 2000; Taub, 2004). The use of ALA and other photosensitizers for various skin conditions—such as acne, hair removal, warts, and psoriasis—is promising, but will require FDA approval following clinical trials (Allison *et al.*, 2004c).

Although oncological therapy has been a force behind PDT, in reality, PDT is much more widely used for non-oncological conditions. Based on well-designed worldwide trials, the photosensitizer Visudyne™ (Verteroporfin; QLT Phototherapeutics, Inc., Vancouver, BC, Canada) obtained FDA and worldwide approval in 1999 for the treatment of the wet form of age-related macular degeneration (Houle and Strong, 2002; Kozak *et al.*, 2006). This disease, characterized by non-controlled neovascularity, is a leading cause of progressive blindness, and affects millions worldwide. The company that has marketed this therapy is also working on oncological PDT in the eye.

(4.2) Current Controversies in PDT

While recent advances in the clinical use of PDT for the treatment of a variety of superficial tumors clearly demonstrate the potential of PDT, this approach has progressed slowly. In some specialties of medicine, such as dermatology, oncology, and ophthalmology, PDT is used frequently, while in others its use remains marginal. Why has PDT not achieved more sustained access to oncological therapy?

Multiple answers may be given to this question: (i) the difficulty in establishing the optimum conditions for a treatment that has several components, (ii) clinician and hospital resistance to a new approach, (iii) the cost of setting up a PDT center, and (iv) the previous lack of inexpensive and convenient light sources. Moreover, to establish clear advantages over alternative treatments, the existing photosensitizers and light sources have to be evaluated in large controlled, comparative, randomized clinical trials.

A significant impediment in the development of clinical PDT in the United States is the difficulty in obtaining FDA approval (on-label treatment). Without FDA approval, insurance reimbursement is difficult to achieve, and this leaves both the provider and the patient with the potential of significant bills. With a few exceptions, the off-label treatment is relatively rare, often leaving the US behind the rest of the world. The approval for PDT should be sought for multiple indications at the same time, because the actual PDT procedure is essentially similar for each anatomical area. Instead, FDA approval of PDT was sought for specific clinical indications. Additional complications in the US originated from manufacturers seeking approval for the drug and device combination. Discussions between manufacturers and different FDA committees have delayed the progress of PDT and bankrupted many small companies (Dougherty, 1996). Although more recent applications to the FDA for new PDT indications are less complicated, "the cost, paperwork and time required for the FDA approval often keeps clinical PDT in the US a step behind" (Allison *et al.*, 2004c).

(4.3) Addressing Current Limitations in PDT

The clinical simplicity of drug-, light-, and oxygen-based reaction has stimulated the current expansion of PDT. A sensitizer is delivered into a patient; the tumor bed is properly illuminated, resulting in apoptosis and tumor necrosis with vascular cessation. Yet even the best currently available systemic photosensitizers accumulate to a certain degree in other organs, particularly in the skin, causing prolonged photosensitivity after exposure to light. Thus, an ideal photosensitizer should be administered easily and safely, targeted appropriately, illuminated and activated at clinically useful wavelengths, pain-free, and obtained easily. Current commercially available photosensitizers embrace some, but not all, of these characteristics. The lack of accurate dosimetry and suitable illumination devices, combined with insufficiently defined treatment parameters, has also diminished the success of PDT (Allison *et al.*, 2006). Although PDT was originally considered as a local treatment, limited to sites where the light activates a photosensitizer, it is now realized that PDT can initiate regional and systemic immune responses (Dougherty *et al.*, 1998). Despite all these limitations, the existing photosensitizers and light sources have achieved significant clinical success, allowing PDT to expand.

The future of PDT will depend on the interactions between clinical applications and technological innovations. Allison *et al.* (2006) have described PDT as the therapy that "is truly the marriage of a drug and a light", and, as a result, only interdisciplinary research approaches can overcome all the difficulties and challenges of PDT.

The development of optimal photosensitizers should address the problems of toxicity, mutagenicity, and elimination of the drug from the patient, selectivity and targetability of photosensitizers, dependable activation by an appropriate wavelength of light, sunlight precautions, simplicity of administration, pain-free outpatient therapy, availability, and cost-effectiveness (Allison *et al.*, 2004a). The activation wavelength of the currently available photosensitizers limits the depth of effectiveness of PDT, in the cancer treatment, to ~ 1.5 cm. The development of photosensitizers with longer wavelengths of activation will allow for deeper tissue penetration. Complicated optical properties of tissues and variability in the light source make it difficult to monitor and verify the amount of radiation that reaches the drug. With the limited dosimetry that is currently available, highly active photosensitizers may easily trigger over-dosage, and less active photosensitizers could also have a therapeutic potential.

A successful PDT should overcome the limitations of currently used light sources. In the past, many of the applications of PDT used a laser source for illumination of the target area, particularly for internal use. Currently, non-coherent broadband sources for irradiation of small and/or large external areas are more common. These sources differ from laser sources in their output characteristics, important differences being the large beam size and broad spectral output. Recently, non-laser light sources, such as light-emitting diodes (LEDs), are making an impact on PDT (Allison *et al.*, 2004a; Juzeniene *et al.*, 2004; Pieslinger *et al.*, 2006; Steiner, 2006). The LED devices are small, lightweight, and highly flexible. In addition, LEDs are highly efficient for second-generation photosensitizers, with absorption wavelengths closer to the LED peak emission. The further development of therapy- and cost-effective light sources for PDT would be of benefit for both research and clinical applications. Reliable fiber optics are also a key advance for clinically successful PDT (Panjehpour *et al.*, 2000). Because successful PDT requires homogeneous light distribution, the fibers must undergo rigorous manufacturing procedures. This in itself is an obstacle to PDT, since optimal therapy may require several expensive systems to illuminate a variety of photosensitizers, and different fibers are not always available, are very expensive, and are not FDA-approved.

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