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Anti-microbial photodynamic therapy: useful in the future?

Abstract Previous chapters in this volume have focused on fundamental principles and clinical applications of PDT. This chapter will attempt to outline emerging areas of research to identify some new applications that may become useful in the future in clinical practise. The worldwide rise in antibiotic resistance has driven research to the development of novel anti-microbial strategies. Cutaneous diseases caused by MRSA are ideally suited to treatment by anti-microbial photodynamic therapy for eradicating localized infections and for modulating wound healing due to the ability to deliver photosensitizer and light with topical application. The use of photosensitizer and light as an anti-microbial agent against periodontal microbial biofilms should also represent an attractive method of eliminating oral bacteria. Suitable light sources, laser light and non-coherent light will be briefly covered. This chapter will focus on some aspects of anti-microbial photodynamic therapy that appear to be promising for dermatological indications and inactivation of pathogenic bacteria within the oral cavity.

Keywords Anti-microbial photodynamic therapy · Oral bacteria · MRSA · Photosensitizer · Porphyrin-photosensitization

Introduction

The phototoxicity of chemical compounds towards microorganisms was first published at the turn of the 20th century. Oskar Raab observed that the toxicity of acridine hydrochloride against *Paramecia caudatum* was dependent on the amount of light, which was incidental on the experimental mixture [64]. In addition, his teacher Hv

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Tappeiner, reported that the toxic effects in the presence of light are not due to heat [82]. After further experiments to exclude direct influence of light, Hv Tappeiner coined the term "photodynamic reaction" in 1904 [83]. Additional investigations demonstrated the involvement of oxygen in killing the bacteria because the anti-bacterial activity of fluorescent dyes against the facultative anaerobic species *Proteus vulgaris* could not be demonstrated in the absence of oxygen.

Photodynamic inactivation of mircoorganisms is based on the concept that a dye, known as a photosensitizer (PS), should be localized preferentially in the bacteria and not in the surrounding tissues or cells, and subsequently activated by low doses of visible light of an appropriate wavelength to generate free radicals or singlet oxygen that are toxic to target mircoorganisms.

Since the middle of the last century, anti-microbial photodynamic therapy was forgotten because of the discovery of antibiotics. Certainly, in the last decades the total worldwide rise in antibiotic resistance has driven research to the development of new anti-microbial strategies. In particular, staphylococcal resistance to methicillin and closely related penicillins was noted since the introduction of penicillinase-stable β-lactam antibiotics like methicillin or cloxacillin [61]. The prevalence of methicillin-resistant Staphylococci strains (MRSA) are increased from less than 5% in the year 1980 up to 20.7% in the year 2001 in Germany [44]. The appearance of MRSA gaining new resistance against vancomycin in the year 2000 further aggravates this problem [26, 74]. As a consequence, infections with MRSA can be difficult to treat and infected patients may be colonised for many months and require long hospital stays [10]. Accordingly, the current treatments range from local disinfectants to systemic antibiotics [60, 73, 87].

Furthermore, the use of antibiotic drugs is not underestimated with respect to resistance issues of e.g. periodontal bacteria [75, 90]. Insufficient drug concentrations within the sulcus fluid or biofilm may also be responsible for the lacking efficacy in killing the bacteria [1, 68]. In addition, MRSA may also develop cross-resistance to

triclosan, an antiseptic used in toothpaste and mouth rinse [15].

The purpose of this review will focus on some aspects of anti-microbial photodynamic therapy to identify some applications that may become useful in the future in clinical practise.

Mechanism of action of photodynamic inactivation of microorganisms

Upon irradiation with light of an appropriate wavelength, the photosensitizer undergoes a transition from a low-energy ground state to a higher energy triplet state. This triplet state photosensitizer can react directly with biomolecules to produce free radicals and/or radical ions (type I reaction), or with molecular oxygen to produce highly reactive singlet oxygen (type II reaction).

Various studies showed that there is a difference in susceptibility to anti-bacterial PDT between gram-positive and gram-negative bacteria [53, 56, 62]. Anionic and neutral photosensitizers were found to bind efficiently to gram-positive bacteria to induce growth inhibition or killing by visible light, whereas gram-negative bacteria were not killed. Growth inhibition of *E. coli* by porphyrin-photosensitization was possible only in the presence of membrane disorganising substances, e.g. the nona-peptide polymyxin or Tris–EDTA [62]. However, direct photo-killing of gram-negative bacteria is also possible. In recent years, different chemical classes of positively charged PS, including phthalocyanines and porphyrins, were successfully tested as photoinactivating agents against gram-positive and gram-negative bacteria so far [47, 54–56, 72]. In general, photosensitizers with an overall cationic charge and meso-substituted cationic porphyrins and water-soluble cationic zinc phthalocyanines can efficiently kill gram-negative bacteria by photosensitization even in the absence of additives. This resistance of gram-negative bacteria against efficient killing by anti-bacterial photodynamic therapy is due to the different outer membrane structures of gram-positive and gram-negative bacteria, which is discussed in detail elsewhere [48].

Inactivation of *S. aureus*, *E. coli* and *P. aeruginosa* is accompanied by alterations of the ultra-structure of the cells, e.g. disordered cell wall structure; elongated cells connected together without separation of the daughter cells and different low density areas in the cytoplasm [49, 62].

However, there is some evidence that treatment of bacteria with PS and light leads to DNA damage [52], even though the modification of this target may not be the prime cause of bacterial cell death because *D. radiodurans*, which is known to have a very efficient DNA repair mechanism, is easily killed by photosensitization [70].

Light sources for photodynamic inactivation of microorganisms

The treatment of bacterial infectious diseases requires that sufficient light intensity is delivered at the level of the photosensitiser-loaded pathogens located either in the subgingival areas or in the skin. In general, the intensity of light decreases with penetration depth through the various skin layers due to the combined effects of scattering and absorption because the skin is irregularly shaped, inhomogeneous, multilayered, and contains hair follicles and glands [86]. The penetration of light into most biological tissues increases upon increasing the wavelength, at least in the 400–700 nm range. Therefore, a compromise must be found regarding the penetration depth of the light, the absorption spectrum of the sensitizer used and the localisation sites of the pathogens. With respect to phenothiazines (methylene blue, toluidine blue) or porphyrins, there is a still effective absorption of light for wavelengths above 600 nm. At the moment, different laser systems and incoherent light sources are used in PDT [3].

Anti-microbial photosensitizing agents

A large number of compounds with photodynamic activity are now available (Table 1). First of all, the naturally occurring photosensitizers have demonstrated photosensitising ability. Psoralens (furanocoumarins) and perylenequinonoid represent just two examples of natural products, which originally act in plants as chemical defence substances against microbial or eukaryotic organisms. In fungi, furanocoumarins normally facilitate the parasitization of plants. Another group of dyes are the synthetic non-porphyrin compounds, like the phenothiazine dyes: methylene blue and toluidine blue. Next, macrocyclic molecules showed phototoxicity, like phthalocyanines and the metal containing porphyrines and the metal-free porphyrines [88].

Table 1 Overview of photosensitizers displaying anti-microbial photocydal action

Class of compounds	Name	Site of action in prokaryotic cells	References
Natural products	Furanocoumarin	DNA intercalation	[31]
	Perylenequinonoin hypericin	Inhibitor of protein kinase C	[23]
Phenothiazines	Methylene blue	DNA interaction	[52]
	Toluidine blue	Plasma membrane	[89]
	Acridine	DNA interaction	[88]
Cyclic tetrapyrroles	Phthalocyanine porphyrine	Membrane/cytosolic sites	[4]

Anti-microbial photodynamic effects against sensitized pathogens with PS

Inactivation of ubiquitous species of *Staphylococcus aureus* was studied using photosensitizers such as haematoporphyrin, phthalocyanine, photofrin and 5-aminolaevulinic acid. The following clinical skin diseases with the involved multi-resistant bacteria species, yeast and fungi should demonstrate the importance of developing possible alternatives for topical and/or systemic antibiotic/ antimykotic therapy.

Treatment of wound infections

Primary topical anti-microbial and antiseptic agents are indicated in both prophylaxis and treatment of infections. One advantage of topical application of anti-microbial agents is their low systemic absorption, consequently the reduced exposure of the commensal gastrointestinal flora to these antibiotics and low systemic toxicity [39]. The principles of anti-microbial treatment of infected skin wounds are discussed extensively by Filius et al. [25].

Today topical therapy with antibiotics has become unpopular because of the development of resistance [94]. Colsky and colleagues made a comparison of antibiotic resistance profiles using data collected from 1992 to 1996 from patients with skin wounds and revealed a marked increase in oxacillin and ciprofloxacin resistance in *S. aureus* and *P. aeruginosa*. In leg ulcers, an increase from 24% to 50% oxacillin resistance in *S. aureus* and from 9% to 24% ciprofloxacin resistance in *P. aeruginosa*. In superficial wounds, an increase from 24% to 36% ciprofloxacin resistance in *P. aeruginosa* [13, 14]. This study demonstrates the rapid increase of antibiotic resistant bacterial pathogens due to the systemic use of antibiotics in dermatology and highlights the importance of searching for alternatives.

In a first report, Hamblin et al. showed the use of a photochemical approach to destroy bacteria infecting a wound in an animal model without damaging the surrounding host tissue [32]. After topical application of a chlorin(e6) photosensitizer conjugated with poly-L-lysine, *E. coli* was rapidly killed upon exposure to selected visible light wavelengths.

Psoriasis

Psoriasis is a multifactorial disease of still unknown aetiology. There are two clinical types of non-pustular psoriasis known: acute guttate psoriasis and chronic type I plaque psoriasis. Bacterial infections such as streptococcal infection are a well-known exacerbating factor in acute guttate psoriasis [28, 35, 50, 67, 94]. In addition, in patients with chronic plaque psoriasis, 50% harbour *S. aureus* on their skin [46]. In addition, not only streptococcal but also staphylococcal superantigens are proposed as a possible antigen in chronic plaque type I psoriasis [63]. Data from Yamamoto et al. suggest that the reactivity of PBMCs to staphylococcal enterotoxin B (SEB) may lead to the exacerbation and persistence of chronic plaque psoriasis by the induction of several inflammatory cytokines [95]. Photochemotherapy (psoralen plus ultraviolet treatment (PUVA)) is a very effective and widely used treatment modality for psoriasis [58, 59]. A disadvantage of such multiple PUVA treatments is the possibility of increasing the risk of developing skin cancer in patients with psoriasis including basal or squamous cell carcinoma or even melanoma [78–80].

Recently, photodynamic therapy with topical application of 5-ALA followed by broadband visible light radiation was tested in patients with chronic stage plaque psoriasis [12, 81]. Selectivity of protoporphyrin IX accumulation in plaque psoriasis after topical application of 5-ALA and photobleaching during PDT was established [81]. However, the clinical response of patients with plaque psoriasis after PDT with topical application of 5-ALA revealed no clear correlation between clearance of plaque areas and the delivered irradiation dose [27, 66]. On the other hand, a study using an ointment containing 10% of 5-ALA, which was applied topically to plaque lesions 5 h before irradiation documented a beneficial effect of PDT in psoriasis [7].

More recently, an open non-randomised phase I and II study in 20 patients with chronic stage plaque psoriasis revealed that after intravenous administration of the photosensitizer verteporfin and subsequent irradiation, all patients exhibited improved clinical response [6].

These preliminary results are encouraging to develop new regimes of systemic application of photosensitizers avoiding an associated prolonged photosensitivity. In the future, the use of PDT with photosensitizer and polychromatic light to treat psoriasis might represent an alternative therapy to PUVA.

Acne vulgaris

Acne is a disease of the pilosebaceous follicles. The principal pathogenic factors in acne are: abnormal follicular keratinisation leading to plugging of the follicle [1]; increased sebum production under the follicular plug [2]; inflammation [3]; proliferation of *Propionibacterium* ssp. in the sebum [4]. *Propionibacterium acnes* and *Propionibacterium granulosum* are found mainly in the sebaceous areas of the skin. However, *P. acnes* is a porphyrin-accumulating bacteria, which can be killed by light without exogenous photosensitizers [40, 41]. At present, the role of these ubiquitous bacteria in the pathogenesis of acne remains unclear because there is a very weak association between the severity of acne and the number of *P. acnes* within superficial pilosebaceous follicles [17]. In contrast, Eady et al. showed that the therapeutic control of acne was lost when *P. acnes* developed resistance to erythromycin [22]. The therapeutic control could be regained when an antibiotic was used against which these bacteria were still sensitive. In vitro experiments revealed that cell wall

extracts and exocellular lipase of *P. acnes* are potent chemoattractants for leukocytes, like neutrophils [45]. Therefore, these bacteria may have an important role in the promotion of inflammatory reactions in vivo. On the other hand, PDT of acne vulgaris with topical 5-aminolaevulinic acid showed an apparent improvement of facial appearance and a reduction in the development of new acne lesions [37]. Recently published reports indicate a selective damage to the sebaceous glands, hair follicles and epidermis [18, 19, 37]. After recovery, a normal skin structure is maintained except for a persistent reduction in the number of hair follicles (decrease in number of pilosebaceous units). Therefore, PDT could be beneficial in the treatment of acne not only by cytotoxic effects on the skin but perhaps by anti-bacterial effects against *Propionibacterium* ssp.

Treatment of superficial fungal infections of the skin

Candida albicans and related species that are pathogenic for man become more and more resistant to traditional antifungals such as fluconazole [38]. A comprehensive overview of investigative studies about the effects of PDT on yeasts and dermatophytes was published elsewhere [9]. The clinical consequences of antifungal resistance can be seen in treatment failures in patients, especially with regards to immunocompromised persons. In HIV, up to 90% of AIDS patients are colonized with fluconazole-resistant *Candida* species receiving therapy for oral candidiasis [38]. Recent in vitro studies showed a susceptibility of *Candida* species to photodynamic effects of photofrin or the porphyrin precursor 5-aminolaevulinic acid after light application [5, 57]. Photofrin was effectively taken up by *Candida* and irradiated organisms were damaged in a drug dose-dependent and light-dependent manner. The susceptibility of *C. albicans* to antimicrobial PDT in vitro, supposes possible applications of this technique prospectively. The first in vivo study demonstrated the efficacy of methylene blue activated with a diode laser for the treatment of oral candidiasis in immunosuppressed mice [85]. Furthermore, PDT induces inflammatory signalling that activates immune competent cells such as macrophages and neutrophil granulocytes, which are involved in the process of killing *Candida* [30].

Helicobacter infection treated by photodynamic therapy

Infections by *Helicobacter pylori* were a causal relationship with gastric ulcer, chronic gastritis and gastric cancer. Since 1994, the International Agency for Research on Cancer (IARC) and the WHO concluded that *H. pylori* has a causal link to gastric carcinogenesis and was categorized as a group I carcinogenic agent in humans (IARC Monograph, 1994, vol. 61). In view of drug resistance, side effects, and compliance and expense of therapy, treatment failure is increasing and new treatment strategies need to be developed [8]. Recently, some attempts to develop anti-bacterial PDT for the eradication of *H. pylori* were successful in vitro only [33]. However, a controlled, prospective trial of endoscopically delivered blue light to eradicate *H. pylori* in regions of the gastric antrum in ten patients showed an overall reduction in *H. pylori* colonies of 91% between treated and control areas [29]. In all cases, no exogenous photosensitizer was applied. Hamblin et al. demonstrated that *H. pylori* accumulate quantities of endogenous coproporphyrin and protopropyhrin IX, which leads to bacteria killing by photodynamic action upon illumination [33].

Photoinactivation of PS-sensitised bacteria using laser light

During the last years, numerous research groups verified the lethal effect of laser radiation on mircoorganisms associated with dental caries, periodontitis and perimplantitis [2, 21, 69]. These studies showed light from both high-power and low-power lasers to be effective in killing oral pathogenic bacteria sensitised with PS in vitro.

Chan and Lai attempted a study to clarify whether the bactericidal effects of phototoxicity are wavelength or dose-dependent to eliminate periodontal pathogens, e.g. *Actinobacillus actinomycetemcomitans*, *Fusobacterium nucleatum*, *Porphyromonas gingivalis*, *Prevotella intermedia*, and *Streptococcus sanguis* [11]. These pathogens were exposed to a HeNe laser (632.8 nm, 30 mW), a 100 mW diode laser (665 nm), a 100 mW diode laser (830 nm), in the presence or absence of methylene blue (MB) as the appropriate photosensitizer. The most effective combination (95–99% bacteria killing rate) was that of MB with a 665 nm diode laser at 100 mW against the various species that were tested (Table 2). The cause of the differing susceptibilities between these species has to be elucidated.

Sigusch et al. used a new dog model to determine the anti-bacterial efficacy of different photosensitizers against two periodontopathogenic bacteria species. All subgingival areas were infected with *P. gingivalis* and *F. nucleatum*. After infection, areas were incubated with chlorin(e6) and irradiated with a diode laser (wavelength 662 nm) using a power of 0.5 W. Chlorin(e6) caused a significant reduction in *P. gingivalis*-infected sites, whereas *F. nucleatum* was hardly reduced.

Another group showed that photodestruction of dental plaque may be a potentially powerful tool for the treatment of chronic destructive periodontal disease using chlorin(e6) or a chlorin(e6) conjugate containing poly-L-lysine residues. Irradiation was done by an argon ion laser [77].

Furthermore, a series of studies showed that it is possible to kill bacteria with low-power laser light when bacteria were sensitised with MB or toluidine blue as the appropriate photosensitizer [42, 71, 92]. Whereas Kojima et al. demonstrated the inhibitory effects of a super pulsed carbon dioxide laser at low energy density on periodontopathic bacteria without any photosensitizer [42].

Table 2 Phototoxicity of oral bacteria sensitised with MB to different laser light

	HeNe (632.8 nm)		Diode (665 nm)		Diode (830 nm)	
	Laser only* (%)	Laser+MB* (%)	Laser only* (%)	Laser+MB* (%)	Laser only* (%)	Laser+MB* (%)
Actinobacillus actinomycetem-comitans	85	13	67	5	52	61
Fusobacterium nucleatum	97	16	61	4	55	50
Porphyromonas gingivalis	102	12	57	0.8	59	42
Prevotella intermedia	98	12	64	0	58	43
Streptococcus sanguis	85	11	60	2	63	44

*Survival of viable count colony forming units. Modified towards [11]

Photoinactivation of microbial biofilms

As mentioned above, a number of studies showed that oral bacteria are susceptible to anti-bacterial photodynamic therapy when they are grown in suspension in vitro [1, 20, 77]. One important pathogenic factor of dental caries and periodontal diseases belong to the existence of microbial plaque/biofilm [16]. A microbial biofilm is generally defined as a community of microorganisms within a polymeric matrix, typically comprising exopolysaccharide. A consequence of biofilm growth and their extracellular products moderates the access to their control in the environment and leads to a large increase in resistance to anti-microbial agents and antiseptics. Current treatment regimes of plaque-related diseases involve effective mechanical removal of subgingival plaque/biofilms and the use of antiseptics and antibiotics. An in vitro study of the use of anti-bacterial photodynamic therapy was carried out for the treatment of natural oral plaque biofilms formed in vivo using toluidine blue combined with a red light-emitting diode (620–660 nm) [96]. A killing efficacy of 95% to 99% was observed after photosensitization of biofilms containing *Streptococcus mutans*, *S. sobrinus* and *S. sanguinis*. Furthermore, confocal scanning laser microscopy of an oral biofilm model showed that a chlorin(e6)-PS was taken up into the biofilm and the combination of light and chlorin(e6)-PS achieved almost 90% killing of the subgingival plaque species [77]. Wood et al. demonstrated that treated biofilms are much thinner than the control samples and show a different structure with little evidence of channels and a less dense biomass [93]. Transmission electron microscopy (TEM) of the in vivo-formed plaque biofilms reveals considerable damage to the bacteria in the biofilm, vacuolation of the cytoplasm and membrane damage being clearly visible after PDT. Therefore, periodontal diseases may be one of the main applications of anti-microbial PDT adjunct to conventional anti-microbial treatments within the oral cavity in the future.

Discussion

Topical anti-microbial treatment must result under critical assessment of utility and risks of related antibiotics: undesirable side effects and the induction of a contact dermatitis or a selection of resistant bacteria strains within the cutaneous microbial colonisation are serious risk factors. Colsky and colleagues made a comparison of antibiotic resistance profiles using data collected from 1992 to 1996 from patients with skin wounds and revealed a marked increase in oxacillin and ciprofloxacin resistance in *S. aureus* and *P. aeruginosa*. In leg ulcers, an increase from 24% to 50% oxacillin resistance in *S. aureus* and from 9% to 24% ciprofloxacin resistance in *P. aeruginosa*. In superficial wounds, an increase from 24% to 36% ciprofloxacin resistance in *P. aeruginosa* [13, 14]. Furthermore, the induction of a contact sensitization was seen in up to 30% of patients with ulcera crurum and 15% of patients with chronic otitis externa after topical application of antibiotics, e.g. neomycin [65]. In addition, Hoiby et al. have demonstrated that ciprofloxacin is eliminated through the sweat after oral use and within the average of 2.7 days after, the resistance of *S. epidermidis* against this antibiotic was induced in the armpit [36]. The development of resistance against erythromycin in 100% of the aerobic bacteria of the skin (above all *S. epidermidis*) after topical treatment, highlights the importance of searching for alternatives [34].

The most suitable application of anti-microbial photodynamic therapy is removed the probability of treating local, superficial skin infections and/or wound infections and the possibility to reduce the nosocomial colonisation of multi-resistant bacteria of the skin. The main factors in successful photodynamic inactivation of pathogens include the optimisation of the side chain chemistry and dosage of the photosensitizer for uptake and penetration, the duration between its administration and light application and the region or extent of body surface area exposed to the activating light may influence the impact of the phototoxicity on mircoorganisms. Recently, Ferro et al. could demonstrate an enhanced inactivation of MRSA by a liposome-delivered photosensitizer compared to the free dye [24]. They used hematoporphyrin embedded in fluid cationic vesicles composed by the monocationic lipid *N*-[1-(2.3-dioleoyloxy)propyl]-*N*,*N*,*N*-trimethylammonium methylsulfate promoting a tighter binding to MRSA. Such a delivery system for anti-microbial PDT could be useful for an enhanced uptake of non-cationic photosensitizers, which show

phototoxicity only in the presence of membrane dis-organising substances [62]. Furthermore, the synergic effect of positively charged PS and highly fluid vesicles can optimise the uptake by bacteria and killing efficacy. In a first report, Hamblin et al. showed the use of a photochemical approach to destroy bacteria infecting a wound in an animal model without damaging the surrounding host tissue [32]. After topical application of a chlorin(e6) photosensitizer conjugated with poly-L-lysine, *E. coli* was rapidly killed.

Neither mechanical plaque removal nor flushing or rinsing with disinfectants allows the complete eradication of bacteria within the periodontal pocket. In aggressive periodontitis or in subjects that are refractory to therapy, treatment with systemic antibiotics is advisable. However, periodontal infections are caused by many diverse bacteria species requiring different antibiotics with different risks of adverse effects [76] Recently, some reviews were published addressing discerningly photodynamic therapy as a new concept for periodontal disease [51, 91]. Furthermore, it was proposed that a photosensitizer can be injected into the periodontal pocket, followed by illumination with fiber optics inserted into the infected area. The advantages of this approach are that bacteria can be eradicated in a very short period of time, resistance development in the target bacteria is unlikely and damage to adjacent host tissues and disruption of the normal microflora can be avoided.

During the last 10 years some major advances in the field of anti-microbial PDT were made, which are characterized by the following points:

– An overall short time of anti-microbial PDT is due to a very fast uptake of the photosensitizer agents by bacteria (few minutes) followed by a relatively low intensity (e.g. 40–100 mW/cm^2) yielding a significant reduction of pathogens (>3 log$_{10}$ reduction of growth curves).
– Availability of a broad spectrum of photosensitizers, e.g. phenothiazines, phthalocyanines or porphyrins, which showed a significant anti-bacterial activity against gram-positive and gram-negative bacteria.
– Applicability against antibiotic resistant bacteria independent from their antibiotic resistance pattern. This property is important regarding the repeated treatment of chronic and/or recurrent infections.
– Lack of induction of resistance after multiple treatments. Ongoing studies showed that at least up to 15 generations of porphycene-photosensitised *S. aureus* and *E. coli* developed no resistance to PDT (Jori, unpublished results).
– Lack of mutagenicity. One potential advantage of PDT over UVA-treatment is that PDT may not be intrinsically carcinogenic.

Topical application of anti-microbial PS needs an appropriate formulation to reach bacteria interepidermally. Therefore, the effects of formulations such as creams, emulsions, lotions, nanocolloids and ointments are essential on the penetration and/or accumulation of these PS and

demand further investigations. Recently, Tegos et al. demonstrated that a new class of PS, named cationic fullerenes, which are more effective and selective anti-microbial PS than the widely employed PS toluidine blue [84]. Fullerenes consist of 60 carbon atoms arranged in a soccer ball-shaped structure.

In summary, the formulation, pharmacokinetics, and the type of PS, the duration between its administration and light application and the region or extent of the body surface area exposed to the activating light may influence the impact of PDT on mircoorganisms relevant in dermatologic diseases. Photodynamic treatment was also proposed as a possible new method for protecting foods from microbial spoilage [43].

Conclusion

The worldwide increase in antibiotic resistance among different classes of gram-positive and gram-negative bacteria has led to search for alternative anti-microbial therapies, like anti-microbial PDT. At this time, there is no routine application of anti-microbial PDT in the treatment of localized infections in such areas as skin, wounds and periodontal pockets. However, if the resistance against antibiotics may become worst, anti-microbial PDT may be an alternative therapy option in clinical practise depending on the pharmacokinetics and the illumination time.

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