Light based anti-infectives: ultraviolet C irradiation, photodynamic therapy, blue light, and beyond

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Abstract

Owing to the worldwide increase in antibiotic resistance, researchers are investigating alternative anti-infective strategies to which it is supposed microorganisms will be unable to develop resistance. Prominent among these strategies, is a group of approaches which rely on light to deliver the killing blow. As is well known, ultraviolet light, particularly UVC (200–280nm), is germicidal, but it has not been much developed as an anti-infective approach until recently, when it was realized that the possible adverse effects to host tissue were relatively minor compared to its high activity in killing pathogens. Photodynamic therapy is the combination of non-toxic photosensitizing dyes with harmless visible light that together produce abundant destructive reactive oxygen species (ROS). Certain cationic dyes or photosensitizers have good specificity for binding to microbial cells while sparing host mammalian cells and can be used for treating many localized infections, both superficial and even deep-seated by using fiber optic delivered light. Many microbial cells are highly sensitive to killing by blue light (400–470 nm) due to accumulation of naturally occurring photosensitizers such as porphyrins and flavins. Near infrared light has also been shown to have antimicrobial effects against certain species. Clinical applications of these technologies include skin, dental, wound, stomach, nasal, toenail and other infections which are amenable to effective light delivery.

1. Introduction

The rising problem of antibiotic resistance has led to fears that medicine will return to the situation of a century ago when extensive wounds and surgery often led to death due to uncontrollable infection [1]. These fears have in turn spurred a major research effort to find alternative antimicrobial approaches which, it is hypothesized, will kill resistant micro-organisms while being unlikely to cause resistance to develop to themselves. At the present time many international research efforts to discovery new antimicrobials are underway. Recently, the emphasis is on how to take precautions against creating, and if possible eliminate, multidrug resistance in concert with exploring new methods to kill pathogenic microorganisms. Karen *et al.* recently pointed out that the investigation of novel non-antibiotic approaches, which can prevent and protect against infectious diseases should be encouraged, and should be looked upon as a high-priority for research and development projects [2].

Prominent among novel non-antibiotic approaches is likely to be the group of light-based technologies, including ultraviolet C (UVC) irradiation therapy, photodynamic therapy (PDT), blue light therapy and other light-based therapies. The most attractive advantages of light-based antimicrobial therapies lie in their ability to eradicate microbes regardless of antibiotic resistance, and the fundamental improbability of the microbes themselves developing resistance to these light based therapies due to the rather non-specific nature of the targets.

In this review, we will provide an overview of recent advances in exploration and understanding of light-based anti-infective therapies, including UVC, PDT, blue light and other promising light-based therapies. To our knowledge, this is the first time that there has been a review covering all these light-based therapies for infectious disease

2. Antimicrobial efficacy of UVC irradiation

2.1 Introduction

UV irradiation (wavelength: 100–400 nm) is electromagnetic irradiation, which is divided into four distinct spectral areas including: UVA (315–400 nm), UVB (280–315 nm), UVC (200–280 nm) and vacuum UV (100–200 nm) (Fig 1) [3]. Among these wavelength ranges, UVC has the best potential ability to inactivate microorganisms because the wavelength of 250–270nm, is strongly and mainly absorbed by the nucleic acids of microbial cells and, therefore is the most lethal range of wavelengths [4]. The bactericidal mechanism of UVC is to cause damage to their RNA and DNA, which often leads to the formation of dimers between pyrimidine residues in the nucleic acid strands. The consequence of this modification is that the production of cyclobutane pyrimidine dimers (CPDs) causes deformation of the DNA molecule, which might cause defects in cell replication and lead to cell death afterwards.

Because tissue penetration of light is limited by the extremely short wavelength and difficulties in the delivery approach, UVC for anti-infective diseases is likely to be applied exclusively to superficial infections. Studies that have investigated UVC inactivation of antibiotic-resistant bacteria have found them to be as equally sensitive as their naive counterparts [5].

2.2 UVC irradiation for bacterial/fungi infections *in vivo*

Reports have demonstrated that UVC efficiently inactivated methicillin-resistant *Staphylococcus aureus (MRSA),* vancomycin-resistant *Enterococcus faecalis (VRE),*

antibiotic-susceptible strains of *E. faecalis* and *S. aureus* [5], Enterococci species, *Streptococcus pyogenes* [6], *Escherichia coli* and *Pseudomonas aeruginosa in vitro* [7]. UVC irradiation was able to disinfect catheters by destroying associated bacterial biofilms, including coagulase-negative *Staphylococcus, Streptococcus, E. coli, E. faecalis, P. aeruginosa, Coryneforms* and so on [8].

UVC also inactivated 3–5 $\log_{10}$ of dermatophyte suspensions *in vitro*, including *Trichophyton mentagrophytes, Trichophyton rubrum, Microsporum canis* and *Epidermophyton floccosum* [9]. When the inactivation efficacies of UVC were compared on pathogenic microorganisms, including bacteria (*P. aeruginosa* and *Mycobacterium* abscessus) and fungi (*Candida albicans, Aspergillus fumigatus*), in both mixed suspensions and single suspensions *in vitro,* it needed much higher fluences to kill fungi than bacteria [10]. The diverse structural features of the cell walls of bacteria and fungi are the main reason for the different killing rates. Different devices that deliver UVC with different power densities also induced different outcomes. More details about UVC germicidal efficacies are presented in Table 1.

One of the key questions about the use of UVC as an anti-infective is the following [3]: Can UVC destroy microbial cells in tissue without causing irreversible and unacceptable severe damage to the host DNA and host cells? In an animal model infected by fungi with third-degree burn, *C. albicans* bioluminescence was showed a significant reduction by UV irradiation. The efficacy of fungicidal by UV was superior to using nystatin cream, one of topical antifungal drug [11]. Another animal study showed UVC irradiation (2.59 J/cm^2 for burns and 3.24 J/cm^2 for abrasions) significantly reduced *Acinetobacter baumannii* burden in UVC-treated wounds by approximately 10-fold compared with non-treated groups (p = 0.019 for burns, p = 0.004 for abrasions) [12].

2.3 Effects of UVC irradiation on host cells and tissues

It is well known that prolonged and repeated exposure to UV irradiation can damage host cells and be particularly hazardous to human skin. As to long-term UVC irradiation of human skin, it is also known to have potential carcinogenicity [13]. When UVC irradiation is applied to treat localized infections, one must consider the possible side-effects of UVC delivered at effective antimicrobial fluences on normal mammalian cells and tissue. The safety issue of UVC germicidal treatment requires that the pathogenic microbe is selectively eradicated while the normal tissue cells are spared.

Sosnin *et al.* convinced the UVC fluence that led to necrosis in Chinese hamster ovary cells, one type of fibroblasts, was more than ten-times higher than that used for killing of *E. coli in vitro* [14]. Dai *et al.* investigated UVC inactivated nearly all bacteria (99%) in suspensions over keratinocytes in confluent monolayer cultures, while it only reduced approximately 6% viability of keratinocytes [15]. Another study demonstrated that UVC selectively inactivated 2 logs (99%) of *C. albicans,* while only approximately 0.77 $\log_{10}$ (18.9%) of keratinocytes was killed at the same UVC fluence [11]. Dean *et al.* found that no significant adverse effects were induced in human primary corneal epithelial cells when the cells were exposed to 1.93mJ/cm^2 UVC (265nm), which induced 100% inhibition of growth of all the bacterial species cultured on agar plates [7]. Mohr *et al.* used UVC to reduce pathogen contamination of platelet concentrates [16]. The results showed UVC inactivated more than 4$\log_{10}$ Gram-positive *S. aureus, Bacillus cereus* and *S. epidermidis,* and Gram-negative *E. coli, P. aeruginosa* and *Klebsiella pneumoniae.* However, UVC irradiation caused mild damage to platelet and leaded to more enhanced platelet metabolism, including enhancing glucose consumption, lactate accumulation and a stronger decrease in pH during storage.

In a study using a murine model, Dai *et al.* demonstrated that the mouse skin tolerated the UVC irradiation at the effective fungicidal fluence. Although UVC caused mild wrinkling of the skin at 24h after light exposure, it showed full recovery in the following days [11]. In order to evaluate the carcinogenicity of UVC, one animal study using hairless mice determined that UVC was less carcinogenic than UVB [17].

Most of the experimental results mentioned above suggest that UVC at appropriate fluences does not cause significant damages to host cells and tissues. However, UVC irradiation still has potential to induce nonspecific damage. Studies demonstrated that the DNA of mammalian cells could indeed be damaged by UVC at its effective antimicrobial fluences. Fortunately however, at the same time, the DNA repairing enzymes of the host cells could rapidly repair the damaged DNA [3]. In addition, to minimize the UVC-induced non-specific damage, the intact skin around the area to be treated could be shielded from UVC illumination. On the other hand, application of UVC is limited in some special locations due to its detrimental effects such as infections of the eyes [18].

2.4 The clinical application of UVC irradiation for infectious diseases

A few clinical studies have demonstrated the antimicrobial effect of UVC irradiation, including the disinfection of cutaneous wounds, promotion of wound healing in non-healing infected leg ulcers by increasing granulation tissue [19,20]. A study presented by Taylor *et al.*, reported that the mean bacterial CFU in joint arthroplasty surgical wounds was reduced by 87% with 0.1 mW/cm^2 ($P < 0.001$) and 92% with 0.3 mW/cm^2 ($P < 0.001$) of UVC [21]. Thai *et al.* used UVC irradiation to treat cutaneous ulcers infected with MRSA [22]. In all three patients, UVC treatment reduced the bacterial burden in wounds and promoted wound healing. Two patients had complete wound closure following 1 week of UVC treatment. Another trial was carried out by the same investigators in 22 patients with chronic ulcers manifesting at least two signs of infection and critically colonized with bacteria. The patients received a single UVC treatment and demonstrated significantly reductions of the bacterial burden [23]. Boker *et al.* enrolled thirty patients with mild-to-moderate toenail onychomycosis to treat with UVC. Improvement by at least 1 measurement point was achieved in 60% of patient at 16-week follow-up compared with baseline. There were some unusual and slight side effects such as temporary mild eythema of the treated toe [24]. In addition to the inactivation of microbial cells in the cutaneous wound, UVC exposure is beneficial for wound healing by promoting the expression of basic fibroblast growth factor (bFGF) and transforming growth factor- [25], although the exact mechanisms of UVC for wound healing is still unclear. Shimomura *et al.* investigated the prophylactic efficacies of UVC irradiation in 18 cases of catheter exit-site infections [26]. Although five cases remained unchanged, ten cases (55%) became culture negative and a further three cases showed a microbial decrease.

In summary, it has been known during the past one-hundred years that UVC irradiation is highly bactericidal; however, using UVC illumination for the prophylaxis and treatment of localized infections is still at very early stages of development. Most of the studies are limited to *in vitro* and *ex vivo* levels, while *in vivo* animal studies and clinical studies are much rarer. A major advantage of using UVC over antibiotics is that UVC can eradicate resistant and pathogenic microorganisms much more rapidly without any systemic side-effects. UVC may also be much more cost effective than the commonly used antibiotics.

3 Photodynamic therapy as an anti-infective

3.1 Introduction and mechanism of photodynamic therapy

Another prominent member of the light-based therapies is known as antimicrobial photodynamic inactivation (PDI) or photodynamic therapy (PDT). PDT can direct injury to

the target cells and tissues by producing reactive oxygen species (ROS) induced by the appropriate wavelength of visible light interacting with pre-applied photosensitizer (PS) drugs. The PS can be specifically activated by light of certain wavelength to its excited state. Interaction of the long-lived excited triplet state of the drug or dye with the ground state of molecular oxygen (also a triplet) results in development of ROS such as singlet oxygen ($^1O_2$) through energy transfer and hydroxyl radicals (HO•) through electron transfer. The formation of ROS and $^1O_2$ can chemically attack a very wide range of biomolecules [27–30] (see Fig 2). The high selectivity of PDT for rapidly growing and hyperproliferating cells suggested it could be useful for selective inactivation of microorganisms which possess very fast growth rates [31]. Recently, PDT has been applied as a discovery and treatment alternative approach for localized infections, which represents an emerging new field [32]. This development has been motivated by the relentless growth in antibiotic resistance now found in all classes of microbial cells and in all countries of the world. The broad-spectrum and rapid killing effect of PDT is particularly appealing in this regard [33].

3.2 Antibacterial efficacy of photodynamic therapy

Bacterial cells can be divided into two groups called Gram-positive and Gram-negative bacteria, characterized by large differences in the cellular structure and organization (Fig 3A and B).

Gram-positive and Gram-negative bacteria have diversity in their three-dimensional architecture and outer cell wall. In Gram-positive bacteria the outer wall (15–80nm thick) demonstrates a relatively high degree of porosity which allows the most commonly used PSs to readily diffuse into the inner plasma membrane [34]. On the contrary, in Gram-negative bacteria, the outer wall possesses a highly organized compact structure which acts as a permeability barrier and prevents diffusion to the inner plasma membrane for most high molecular weight compounds [35]. The different permeability barriers between Gram-positive and Gram-negative bacteria are responsible for the observed sensitivity differences when treated with many antimicrobial agents. In principle, PDT has been shown to be more effective against Gram-positive than Gram-negative, especially when neutral or anionic PSs were used [31]. However, non-cationic PSs only bind to the outer membrane of Gram-negative bacteria cells, and do not inactivate them after illumination since the ROS is not produced in sensitive regions. The molecular structures of a range of PSs used for antimicrobial applications are shown in Fig 4.

A number of PSs, including cationic substituted Zn(II)-phthalocyanines [36] (Fig 4A), poly-S-lysine-porphyrin conjugates [37] (Fig 4K), meso-tetrahydroporphyrin, tetrahydroporphyrin-tetratosylat (THPTS) [38,39] and cationic water-soluble gallium(III) phthalocyanines (GaPcs) [40] (Fig 4B), can substantially reduce MRSA populations (4–5 $\log_{10}$) after illumination. Moreover, it has been shown that a PS conjugate of polyethylenimine and chlorin(e6) (pEI-ce6) (Fig 4I) irradiated with red light is capable of reducing MRSA CFU by 2.7 $\log_{10}$ in a murine skin abrasion model [41]. Methylene blue (MB) mediated PDT inactivated VRE [42], and vancomycin-porphyrin conjugates were able to inactive *in vitro* vancomycin-sensitive and VRE [43]. Cationic PSs such as pL-ce6 and MB could destroy the protective effects of extracellular slime and stationary bacterial growth phase when PDI was used to inactive isogenic pairs of wild-type and mutant *S. epidermidis* and *S. aureus* [44].

Multidrug-resistant (MDR) and pandrug-resistant (PDR) Gram-negative bacteria are less prevalent than MRSA but bring about an equally grave threat of truly intractable infections [45,46]. In a model study, PDT also showed high efficiency to anti-infection of Gram-negative bacteria. Sixty MDR *P. aeruginosa* isolates were inactivated by toluidine blue O (TBO) (Fig 4C) mediated PDT with up to 6–7$\log_{10}$ reductions in CFU [47]. This study also

demonstrated that antibiotic-resistant *P. aeruginosa* was just as sensitive to PDI as antibiotic-susceptible strains. Another report showed that MDR strains of *Aeromonas hydrophila* were inactivated by PDT mediated by cationic phthalocyanines [48]. Using 5-phenyl-10,15,20-tris(N-methyl-4pyridyl)-porphine trichloride (also known as Sylsens B or Tri-P(4)) (Fig 4E) as the PS, Trannoy *et al.* obtained a PDI reduction of *Yersinia enterolitica* CFU by 5 $\log_{10}$ [49].

MDR and extensively drug-resistant (XDR) strains of *Mycobacterium tuberculosis* (MDR-TB and XDR-TB) are a rising threat in the developing world which requires long term treatment. Infections have serious adverse effects, are difficult to cure and even fatal [50]. PDT studies on TB have been focused on the homologous system *M. bovis* Bacille Calmette Guerin (BCG) both *in vitro* applying phenothiazinium salts and in murine models of localized mycobacterial induced granulomatous formation [51,52]. In a similar fashion, the antimicrobial effects of PDT have been assessed on infections caused by *M. marinum* [53] and on rapidly growing nontuberculous mycobacteria keratitis [54].

Recently, some novel designed and synthesized PSs have been explored for anti-infective therapy with PDT such as the closed all-carbon cages called fullerenes. $C_{60}(>ME_1N_6{}^+C_3)$ and $C_{60}(>ME_3N_6{}^+C_3)$ (Fig 4L), which are two analogous pentacationic [60] fullerenyl monoadducts with variation of the methoxy-ethyleneglycol length, exhibited high activity for targeting and PDI Gram-positive and Gram-negative bacteria [55]. Polycationic chitosan-conjugated rose bengal (CSRB) as PS showed anti-biofilm ability on *Enterococcus faecalis* (Gram positive) using PDT [56]. Temoporfin liposomes conjugated with a specific lectin (wheat germ agglutinin, WGA) on the liposomal surface eradicated all MRSA by PDT compared with non-modified liposomes [57]. A new PS integrated a potent second generation photosensitizer (temoporfin) and a novel antimicrobial peptide (WLBU2) into liposomes that killed all MRSA upon illumination with 652 nm laser (1 W/cm^2, 100 s, 100 J/cm^2) [58]. Tsai *et al.* showed that 0.25 μM micellar hematoporphyrin (Hp) completely eradicated *S. aureus* under a light fluence of 50 J/cm^2 [59]. The potentiating bactericidal effect of chitosan on Hp- or TBO-mediated PDI was also found with other Gram-positive bacteria (*S. pyogenes* and *S. epidermidis*) and two Gram-negative bacteria (*A. baumannii* and *P. aeruginosa*) while causing less damage to human normal tissues [60]. Tetracationic meso-arylsubstituted porphyrin (RM24) induced PDI can completely eradicate cultures of 10^8 CFU/mL *P. aeruginosa* PAO1 in stationary phase [61].

Moreover, PDT demonstrated significant killing effects on some bacteria which are very difficult to inactivate by antibiotics. For example, Hypocrellin A (HA)-mediated PDT significantly reduced the survival rate of *Bacillus subtilis* to less than 0.1% (*P*<0.05) [6]. Walther *et al.* reported that protochlorophyllide mediated PDT inactivated *Listeria monocytogenes* and *Bacillus subtilis,* while *Yersinia pseudotuberculosis* were found to be insensitive to protochlorophyllide treatment [62]. However, the two bacteria were showed susceptibility to eradication by protochlorophyllide (10 mg/L) combined with polymyxin B nonapeptide at 50 and 20 mg/L, respectively.

In addition to *in vitro* studies, several *in vivo* studies have been carried out to investigate and improve the efficacy of antimicrobial PDT in animal models that mimic clinical human infections. Different kinds of wounds such as burns, abrasions, soft tissue disruption, and excisional wounds have been investigated. These wounds are easy to infect and frequently involve Gram-negative as well as Gram-positive bacteria. Hamblin *et al.* first investigated the effect of PDT *in vivo* using mouse excisional wound models infected with *P. aeruginosa* and *E. coli,* respectively [63,64]. A light dose dependent response was observed in bacteria killing. Moreover, only the animals treated with PDT survived in *P. aeruginosa* infected wounds. PDT using TBO significantly inactivated *Vibrio vulnificus* that could otherwise

lead to fatal sepsis. By using a mouse model of burn infected with *Acinetobacter baumannii*, Dai *et al.* found that, PEI-Ce6 mediated PDT produced 1.7–3 $\log_{10}$ reduction of bacteria while it did not inhibit the wound healing [65]. In a soft tissue model developed by intramuscular injection of bacterial suspension into the mouse thigh muscle, Berthiaume *et al.* studied the effect of PDT against *P. aeruginosa* using bacteria mixed *in vitro* with the tin (IV) chlorin e6—monoclonal antibody conjugate and shining the light after injection [66]. The significant decrease of bacteria was demonstrated. Hypocrellin B: lanthanum (HB:La^{+3}) mediated PDT was able to reduce multidrug-resistant *P. aeruginosa* burden in burn wounds, inactive bacterial levels 2–3log in blood and delay bacteremia compared with untreated control group. In addition, Hashimoto *et al.* reported the *in vitro* reduction of *P. aeruginosa* [67]. Park and coworkers investigated chlorin(e6)-mediated PDT antimicrobial effects in a mouse model in which skin wounds were infected by bioluminescent *S. aureus* Xen29 [68]. MB-induced PDT produced maximal bacterial killing and minimized killing of host neutrophils in murine MRSA arthritis models [69]. PDT with Na-pheophorbide significantly prevented leg swelling, inhibited bone destruction on osteomyelitis models in rats infected by methicillin-sensitive *S. aureus* (MSSA) [70].

PDT has been widely applied in oral and dental infections where Gram-negative bacteria are really common and are thought to be responsible for the most damaging infections. The use of PDT in different models of peri-implantitis demonstrated a decrease of bacterial burden with different bacterial strains. The viabilities of *A. actinomycetemcomitans*, *P. gingivalis*, and *Prevotella intermedia* bacteria were reduced significantly after TBO mediated PDT [71]. PDT was able to reduce *Fusobacterium sp* and *Prevotella sp* counts [72,73]. Significant results were also obtained in non-surgical treatment of periodontitis in *in vivo* models. PDT using TBO caused a significant reduction of *P. gingivalis* CFU in a rat model [74]. The inactivation of *P. gingivalis* was achieved in infected sites using PDT with chlorin(e6) in a beagle dog model [75]. Bottura *et al.* used a model of immunosuppressed rats to demonstrate the effect of PDT on reducing bone loss resulted in experimental periodontitis [76]. Several more studies were in agreement with the previous literature, and further manifested the effectiveness of PDT in treatment of periodontal disease [77–79]. In order to evaluate the effect of PDT on inflammatory cells in periodontitis, Seguier *et al.* tested two different drug delivery systems [80]. The results demonstrated that PDT suppressed the gingival inflammatory reaction during chronic periodontitis and resulted in a specific decrease of antigen-presenting cell populations based on which drug delivery system was used.

3.3 Antifungal efficacy of photodynamic therapy

The clinical location and visible appearance of fungal infection varies widely, from superficial, subcutaneous to severe systemic infectious disease. Fungal pathogens are variable in their cell envelopes, possessing outer wall formed from mixtures of mannan, chitin polymers, and beta-glucans (Fig 3C). This feature causes them to be inherently more permeable to external substances when compared to Gram-negative bacteria.

The hypothesis that cationic PSs would be more efficient in PDI has been tested in Candida species, which are proposed to be the most common fungal pathogens, by several *in vitro* and *in vivo* studies [81–83]. *C. albicans* were inactivated by PDT using PSs including RB, Photofrin (Porfimer sodium) and Al (III)-tetrasulfonated phthalocyanine [84–86]. In another study, *C. albicans* suspensions were irradiated with helium–neon or gallium-aluminum-arsenide lasers using five different PSs including methylene blue (MB), thionin, crystal violet, toluidine blue O (TBO) and phthalocyanine. Except for phthalocyanine, all other PSs tested resulted in a significant reduction in *C. albicans* CFU following PDT [81]. PDT mediated by Photogem, a porphyrin PS, against four different species of Candida, *C. tropicalis*, *C. albicans* and *C. dubliniensis* were completely eliminated, except *C. krusei* [87]. MB-mediated PDT achieved a drug-dependent photo-killing of oral candidosis infected by

C. albicans in a murine model [88]. Junqueira *et al.* demonstrated that the rats treated by MB-mediated PDT had less candidiasis lesions compared with the untreated control group [83]. On the other hand, Giroldo *et al.* confirmed that MB-induced PDT increased the permeability of cell membrane of *C. albicans*, which in turn could increase the sensitivity of this microorganism to other antifungal agents [89]. Effective killing of azole-resistant strains of *C. albicans* [88,90,91] and *C. glabrata*, [87,91] by PDT was also achieved with the use of TBO [88], MB [90], cationic porphyrin [91] and Photogem® [87] as PSs. However, investigators reported that PDT produced less substantial killing of azole-resistant strains in *Candida* [87,90]. Dovigo *et al.* investigated the PDI of oral candidiasis infected by *C. albicans* in a murine model using curcumin as the PS [92]. Results showed that exposure to curcumin (Fig 4H) combined with light-emitting diode (LED) light caused a significant reduction in *C. albicans* viability following PDT in a PS concentration dependent manner without harming the host tissue of mice. Mima *et al.* investigated that Photogem® illuminated by 455 or 630 nm LED light (305 J/cm^2) inactivated *C. albicans* in a murine model of oral candidosis. The results showed a significant reduction in *C. albicans* recovered from the tongue (P < 0.001) compared control group [93]. Mima *et al.* did another *in vitro* study showed the effectiveness of Photogem® induced PDT for the inactivation of different five species of *Candida* on maxillary complete dentures, including *C. albicans, C. tropicalis, C. glabrata, C. krusei* and *C. dubliniensis.* The results showed PDT reduced 3.99 log$_{10}$ CFU from dentures which were infected all species of Candida [94].

T. rubrum is a common pathogenic fungus genus of dermatophytes. PDT was suggested as a potential treatment modality to kill *T. rubrum* with the use of the broad band white light and PSs, including porphyrins deuteroporphyrin mono-methylester (DP-mme) and 5,10,15-tris(4-methylpyridinium)-20-phenyl-[21H,23H]-porphine trichloride (Sylsens B) [95]. PDT inactivated *T. rubrum* in both suspension culture and its isolated microconidia [96]. Red light showed the higher penetration was suggested to be more effective especially for nail infections by PDT. Results of the study demonstrated that Sylsens B was the better PS against both hyphae and the microconidia of *T. rubrum* which completely inactivated the fungal spores and destroyed the fungal hyphae.

Aspergillus fumigatus can cause opportunistic infections in the lung, sinuses and brain of patients with predisposing conditions [97]. Aspergillosis caused by this fungus is frequently fatal, increasing in frequency, and difficult to treat. The treatment is even more complicated by the increasing incidence of drug-resistant strains. Significant fungicidal effects of PDT on *A. fumigatus* was demonstrated *in vitro*, implying that PDT may be a treatment approach for certain localized fungal infections that form granulomas and can adapt themselves to invasive growth [98,99]. However, in a study performed by Gonzales *et al.* comparing the conidial phototoxicity of *Metarhizium anisopliae* and *A. nidulans* with TBO and MB under various incubation times and light doses, the investigators observed that even though conidial killing rates reached up to 99.7%, and germination of the surviving conidia was delayed, none of the treatments achieved a 100% fungicidal effect [100].

3.4 Anti-bacterial/fungi biofilm efficacy of photodynamic therapy

The formation of biofilm is most commonly associated with chronic infections which are typical of microorganisms adhering both to each other and/or to surfaces or interfaces and embedded in an extracellular polymeric matrix. Once biofilm is established, these communities form a protective niche that facilitates growth and survival of bacteria in a hostile environment [101]. The protected environment and the density of the film, as well as the significantly different cellular properties from those in free-floating bacteria of the same species, have been implicated in about 1,000-fold resistance to antiseptics, detergents and antibiotics [102]. Owing to the prevalence of biofilms as a cause of many diseases and the fact that they significantly increase the bacterial resistance to conventional antibiotic

therapies, it is necessary to develop new modalities to treat microbial biofilms. PDI is a possible alternative to inactivate biofilms. Moreover, PDI is a simple non-mechanical therapy and it can be also used for the treatment of children, handicapped people and medically compromised individuals.

S. epidermidis and *S. aureus* are often the pathogens of persistent infections occurring with implanted medical devices that are hard to eradicate [103]. Sharma *et al.* studied the effects of TBO-induced PDT on the structure and the viability of biofilms of *S. epidermidis* and of a MRSA strain [104]. They found PDT significantly reduced staphylococcal biofilms, and the combination of tetrasodium EDTA with PDT enhanced the phototoxic efficacy in *S. epidermidis* biofilms, but not in *S. aureus* biofilms. Zanin *et al.* found that TBO (0.1 mg/ml) induced PDI reduced 2.10–3.11 $\log_{10}$ CFU of *S. mutans* composed in biofilms treated in a dose-dependent manner [105,106]. The average reduction in CFU varied between 1.00 to 2.44 $\log_{10}$ for the biofilms formed by two and three species, and it was verified that TBO-based PDT has a substantial effect on staphylococcal biofilms which can decrease 5 $\log_{10}$ CFU irradiated with red light, disrupt the architecture of biofilm and damage the membranes of bacterial cells. PDT also has an influence on *S. mutans* biofilms in different maturity stages (inactivated 4 $\log_{10}$ CFU), as well as mature *S. sanguinis* and *S. sobrinus* biofilms.

MB-PDT induced an average reduction of 2.81 $\log_{10}$ of *S. mutans* biofilms, as well as an average CFU reduction of 3.29 $\log_{10}$ of *S. aureus* biofilms [107]. Merocyanine 540 (Fig 4J) mediated PDT showed a significant inactivation effect on the survival of *S. aureus* biofilms. However, the light fluences required to inactivate biofilms were much higher than those used to photoinactivate planktonic cultures, due to hindrance of the penetration of light and/ or PS within the biofilms [108]. PDI with the cationic porphyrin, tetra-substituted N-methyl-pyridylporphine (TMP) was effective in biofilm adhering to medical implant surfaces when combined with vancomycin and the host defenses [109]. Tri-meso (N-methyl-pyridyl), meso (N-tetradecyl-pyridyl) porphine (C14) (Fig 4M) presented a significantly greater PDI effect on the eradication of *S. epidermidis* biofilms when compared with the parental tetra-substituted N-methyl-pyridyl-porphine (C1) [110]. Erythrosine (ER) was found to be able to inactivate *S. mutans* biofilms better than protoporphyrin and MB with the inactivation effect enhanced by 2 $\log_{10}$ [111]. Erythrosine induced PDT was also more potent than MB, RB and TBO against *Aggregatibacter actinomycetemcomitans* biofilms [112]. PDI mediated by both 5-ALA and TMP at different concentrations could eliminate *P. aeruginosa* biofilms [113,114]. Recently, Hegge *et al.* investigated the phototoxicity of curcumin in *S. epidermidis* biofilm and suspension cells in two models *in vitro* [115]. The phototoxicity of curcumin towards planktonic *S. epidermidis* was dependent on the presence and type of nanocarrier (PEG 400, HPcCD and F 127). A different situation was observed in a biofilm, where the phototoxicity of curcumin was less sensitive to the selected nanocarrier. Therefore, curcumin should be optimally formulated to target both biofilms and planktonic bacteria in order to obtain maximum photoinactive effect.

Peri-implantitis involves the bacterial colonization, typically in the form of biofilms on the implant surfaces and may lead to system infection and destroy the implant surface. Approximately 20 % of the oral bacteria are streptococci, which are correlative of the dental plaque formation and the development of oral biofilms. *Streptococcus mutans* and *Streptococcus sanguinis* normally exist as regular members of the mature dental biofilm community, and causes caries and periodontal diseases. Pereira *et al.* examined the effect of PDT using ER and Rose Bengal (RB) as the PSs, respectively, and LED on *S. mutans* and *S. sanguinis* biofilms [116]. PDT induced significant decreases in the viability of both microorganisms, especially *S. sanguinis*.

Biofilm formation is also a key event in the development of fungal disease. Similar with the previously mentioned bacterial biofilms, fungal biofilms cause treatment resistance and high mortality, particularly in immunocompromised patients [117]. Candidiasis, caused most commonly by *C. albcans* and to a lesser extent by *C. tropicalis*, *C. parapsilosis* or *C. glabrata*, is often associated with biofilm formation on the surface of medical devices and tissues [118]. Candida biofilm is one of the most common causes of clinical complications found with implanted biomaterials such as intravascular catheters, prostheses, pacemakers, stents, urinary catheters, shunts and orthopedic implants and unfortunately treatment with current antifungal agents are challenging [119]. Candida biofilms resist most of the present antifungal agents, except echinocandins. However, an increase in resistance to echinocandins is now frequently reported [120].

It was revealed that *C. albicans* biofilms were susceptible to photofrin induced PDT [121], and *C. albicans* and *C. dubliniensis* were sensitive to erythrosine-mediated PDT, however the biofilms of both *Candida spp.* were more resistant than their planktonic counter parts [122]. Another study also showed the effects of photofrin (10μg/ml) mediated PDT against *C. albicans* biofilms and its germ tubes [121]. Biofilms treated with PDT showed significant reduction of metabolic activity compared to the biofilms treated with amphotericin B (10μg/ml). The same authors also investigated the effect of PDT against biofilms of *C. albicans* and *C. dubliniensis* using erythrosine (400 mM) illuminated by green LED light (532±10 nm, 237 mW/cm^2, 42.63J/cm^2) [123]. Scan electronic images demonstrated a decrease in the amounts of hyphae and conidia in the biofilm following PDT. However biofilms of both Candida species showed more resistance than their planktonic counterparts. The combination of erythrosine and RB-mediated PDT demonstrated some effect on biofilms, and was also effective in destroying and reducing the blastoconidia and hyphae of *C. albicans* [122]. MB-PDT combined with 660nm laser exhibited a modest reduction in biofilm *C. albicans* species [107]. Mentareva *et al.* demonstrated that the best phototoxic effects on *C. albicans* biofilms were obtained by Zn (II) and Ga (III) phtalocyanines, such as ZnPcMe (Fig 4A) and GaPc2 induced PDT, compared with other diverse PSs [40]. Coleman *et al.* demonstrated that through coupling of saponins with PSs (RB (Fig 4F) and chlorin[e6]), incubating with the yeasts for 30 min and irradiating with visible light, it was possible to inhibit the formation of *C. albicans* biofilm and dramatically potentiate PDI *in vitro* [124]. In one *in vitro* study, Donnelly *et al.* developed a mucoadhesive patch that contained TBO as a PS delivery system for PDT of oropharyngeal candidiasis [125]. This patch was able to resist dissolution when it interacted with artificial saliva and prevented staining of lips, teeth and buccal mucosa. When TBO was released directly into an aqueous sink upon illumination (635nm, 100mW/cm^2, 200J/cm^2), patches were capable of killing *C. albicans* biofilm. Recently, Ribeiro *et al.* evaluated that the effectiveness of chloroaluminum-phthalocyanine (ClAlPc) induced PDT in the treatment of *C. albicans* planktonic cultures and biofilm with different drug delivery system [126]. Cationic nanoemulsions –ClAlPc and free ClAlPc caused significant damage to the fungal cells membrane (P < 0.05); furthermore, cationic nanoemulsions –ClAlPc reduced significantly both cell metabolism and fungal colony counts and also showed better killing rates for Candida biofilms (P < 0.05).

PDT eradication of microbial biofilms could lead to a variety of applications in clinical conditions. For examples, PDT has been used to target to: 1) superficial infections, including elimination of dental plaque, periodonitis, gingivitis, endodontics and oral candidiasis; 2) subcutaneous infections including sinusitis, and osteomyetitis; 3) internal organs infection including endocarditis and the infection of cystic fibrosis; 4) various catheter infections and 5) some temporary or permanent indwelling devices such as joint prostheses, heart valves and other implants, and so on [32]

In conclusion, there is a wealth of literature describing PDT-based anti-biofilm strategies that focus mostly on the use of different PSs against a variety of microbial species. In contrast there are only a limited number of studies exploring the effects of PDT on phenotypic biofilm elements (e.g.,adhesins and extracellular polysaccharide) [127,128]. Moreover, there is lack of standard reproducible models for assessing PDT efficacy against biofilms. The majority of published researches used methodologies where biofilms were grown in/on plastic or silicon microtiter plates and surfaces. These bioassays have been repeatedly criticized for lack of stability and clinical relevance and occasionally yield inconsistent results. For fungal biofilm, it involves a heterogeneous mixture that is composed of hyphae, pseudohyphae and blastoconidia embedded in extracellular polymeric substances which form pores and channels and display diverse phenotypic features compared to planktonic yeasts [129]. The extracellular matrix is composed of proteins, polysaccharides, hexosamine, uronic acid and DNA which promote biofilm formation and adhesion, protecting the cells from phagocytosis [129,130]. This in turn maintains and limits the diffusion of substances, such as antifungal agents, and also restricts penetration of PS and light during PDT [129,130]. Other reasons for increased resistance against both antifungal agents and PDT are the presence of cell wall thickening, up-regulation of drug-efflux pumps, and higher density and larger size of cells in yeast biofilms, decreased number of targets for singlet oxygen per unit of cell volume, existence of multiple species (bacteria and yeasts together), enzymatic alteration of active agent, presence of persisters which represent a subpopulation of cells spontaneously going into a dormant, non-dividing state and in turn remaining alive even after antimicrobial treatments [119,122,129,131,132]. Through modification of the treatment conditions such as incubation time, fluence rate, type of PS and PS' concentration, the efficacy of photo-inactivation can be enhanced especially for resistant cases.

3.5 The efficacy of PDT on other pathogens

Several protozoa are quite dangerous and even deadly human pathogens and most of them are immune to the presently used antimicrobial therapeutic modalities. Typical examples are represented by *Acanthamoeba, Leishmania spp*, *Entamoeba histolytica*, *Plasmodium spp* and *Giardia intestinalis*.

Species of *Acanthamoeba* are facultative parasites, capable of living within the tissues of vertebrates and are the causative agents of granulomatous amoebic encephalitis and amoebic keratitis [133]. The life-cycle of *Acanthamoeba* species are divided into two stages: a dormant cyst and trophozoite, and both stages are infectious. The trophozoitic stage is delimited by a plasma membrane that mainly consists of phospholipids, while the mature cysts' wall is composed of an inner (endocyst) and outer (exocyst) layer separated by a space with a tightly organized structure. The special osmotically inextensible wall, especially endocyst layer, makes the cyst highly resistant to various chemical agents [134]. Limited reports have shown that cationic porphyrins and phthalocyanines, excited by visible light, caused an efficient photosensitized killing of parasitic protozoa. More specifically, tetracationic phthalocyanine (RLP068)-mediated PDT showed the photo-toxicity effect on *Acanthamoeba palestinensis* which almost induced complete inhibition of the viability of *A. palestinensis* in the trophozoite stage [135]. Hypocrellins B (HB) induced PDT manifested effectiveness on *Acanthamoeba* cysts and trophozoites *in vitro*. The results showed HB-PDT induced a total inhibition of the trophozoites and cysts in a dose-dependent manner and a better phototoxic inactivation on the trophozoites than the cysts. However, HB-PDT also manifested phototoxicity on the corneal epithelial cells and stromal cells in the rabbit model [136]. Another study showed that MB-mediated PDT reduced the respiratory activity of *Acanthamoeba castellanii* (ATCC 50370) trophozoites in an MB-concentration dependent manner [137]. Recently, Siddiqui *et al.* used Sn (IV) porphyrin (a synthetic

metalloporphyrin) as PSs illuminated by visible light to treat *A. castellanii* [138]. The results demonstrated that PDT suppressed *A. castellanii* growth, the ability of cytopathogenicity and decrease the adhesion to human corneal epithelial cells in a concentration-dependent manner. However, PDT had no effect on its viability.

Leishmania is an intracellular parasite that parasitizes the phagosomal compartment of macrophages in the vertebrate host and is transmitted to human body via the bite of infected sandflies. Leishmaniasis causes substantial disfigurement and sometimes mortality in the developing countries. The clinical manifestations of Leishmaniasis in humans include cutaneous, mucocutaneous and visceral forms with a wide range of geographic distribution [139]. Recent therapeutic modalities include systemic and topical treatments [140]. However, none of these treatments have been evaluated to be completely satisfactory, and only a few of the touted agents have been proved adequately in clinical trials. Up to now, multiple investigations performed have shown the effects of PDT on killing *Leishmania in vivo* and *in vitro*. Some PSs manifested phototoxicity to inactive *Leishmania* strains, including MB [141], (3,7-bis(N,N-dibutylamino) phenothiazinium bromide (PPA904) (Fig 4D), -aminolevulinic acid-derived protoporphyrin IX (ALA) [142–144], chloroaluminum phthalocyanine encapsulated ultra-deformable liposomes (UDL-ClAlPc) [145], dimethyl and diethyl carbaporphyrin ketals (CKOMe and CKOEt) [146]. PDT caused obvious inactivation on several *Leishmania* strains, such as *L. panamensis*, *L. chagasi* [145], *L. tarentolae* [146], regardless whether their life-stages were promastigotes, axenic or intracellular amastigotes. In some clinical studies, MB-, ALA- or MAL-mediated PDT showed effectiveness to treat cutaneous leishmaniasis. Cosmetic results were excellent and nearly no recurrence occurred after PDT [140,147–149]. Topical PDT showed safety as well as effectiveness, and can be applied as an alternative approach for cutaneous leishmaniasis.

Plasmodium spp are the main causative pathogens of mosquito-borne diseases transmitted from one individual to another resulting in transfusion-transmitted malaria (TTM). There are several infective members of the *Plasmodium* genus, including *P. falciparum*, *P. ovale*, *P. vivax* and *P. malariae* [150]. As a result of increased international travel, migration, and the spread of drug-resistant parasites, malaria is a growing issue in malaria-endemic areas and industrialized nations. Phthalocyanines (one of the most effective, HOSiPcOSi(CH,), (CH,),N(CH,), (Pc 4)), illuminated by red light, manifested the photoinactivation of *P. falciparum* parasitized in red cells in a dose-dependent manner and could be used to make blood components safe [151]. Another study demonstrated that ALA-induced inactivation of plasmodial heme-cycle intermediates, by exposure to white light, reduced *P. falciparum* in culture to levels such that they were not detectable by light microscopy nor by lactate dehydrogenase assay [152].

Human African trypanosomiasis, caused by *Trypanosoma brucei rhodesiense* or *Trypanosoma brucei gambiense* is a disease contracted after biting by the tsetse fly, and is usually a disease involving the nervous system, also known as sleeping sickness. Conversely, American trypanosomiasis, or Chagas' disease, is commonly produced by infection by *T. cruzi* [150]. None of the diseases have an obvious cutaneous appearance (except the puncture wound caused by the biting insect disease vector), but the protozoa exist in the bloodstream of infected persons. Recent studies showed silicon phthalocyanine Pc4, thiazole orange and gentian violet induced PDT manifested photoinaction for *T. cruzi* [153]. Especially, gentian violet -induced PDT has already been used to disinfect *T. cruzi* in donated blood, and this also offers a strong potential lead for conventional drug discovery against *T. cruzi* [154]. In one *in vitro* study, Do Campo *et al.* demonstrated the a 1% solution of MB or 0.001% solution of MB illuminated by white light (100W) immobilized *T. brucei* in citrated guinea pig blood without any subsequent disease when administered to mice [155]. Another study showed that the ultraviolet-absorbing compound Amotosalen (4 -

aminomethyl-4,5 ,8-trimethylpsoralen) when illuminated by UV completely inactivated the infective form of *T. cruzi* both in platelet concentrates and in fresh-frozen plasma [156]. Gottlieb *et al.* inactivated *T. cruzi* trypomastigote forms in the composition of blood by PDT with three phthalocyanine dyes, including HOSiPcOSi(CH3)2(CH2)3N(CH3)2(Pc4), HOSiPcOSi(CH3)2(CH2)3N+(CH3)3I-(Pc5) and aluminum tetrasulfophthalocyanine hydroxide (AlOHPcS4) [157]. The compound Pc 4 was shown to be highly effective in inactivating *T. cruzi*, Pc 5 was less effective and AlOHPcS4 was ineffective. Ultrastructural analysis showed the parasites' mitochondria were a primary target of phthalocyanine dyes-induced PDT.

3.6 The efficacy of PDT on viruses

It has been reported that PDT can also be used to inactivate enveloped viruses. Pristine C60 fullerenes induced photo-inactivation of *vesicular stomatitis virus* (VSV) (Rhabdoviridae) or *Semliki forest virus* (Togaviridae), which produced up to 7-$\log_{10}$ of loss of infectivity [158,159]. Hirayama *et al.* demonstrated that methoxy-polyethylene glycol conjugated fullerene induced PDT, illuminated by white light (120 J/cm^2), and damaged more than 5 $\log_{10}$ of plaque forming units of VSV [160]. Lin *et al.* compared two region-isomers of carboxyfullerene, illuminated with or without light, inactivation dengue-2 and other enveloped viruses [161]. The asymmetric isomer showed greater dark activity, albeit at much higher concentrations than required for its PDT effect. The authors concluded that this difference was attributed to the interaction with the lipid envelope of the virus.

In general, polyomaviruses are considered to be highly resistant to PDI. The degree of resistance is dependent upon the structure of the PS; TBO, acridine orange and MB are effective PSs for PDI of polyomaviruses. Higher doses of PS and light may be required in order to inactivate the oncogenic properties of these viruses than those required to inactivate infectivity [162]. Human papillomavirus (HPV) infection is very common in many populations and manifests several clinical features, including genital warts, verruca vulgaris, verruca plantaris, verruca plana and so on. The clinical applications of PDT have been well reviewed by Lee *et al.* in terms of efficacy, safety and cosmetic outcome [163]. In most clinical cases ALA and methyl aminolevulinic acid (MAL) are used as PSs, even if they are off-label treatments.

3.7 The efficacy of PDT on vectors

Insects act as the crucial roles to disseminate tropical disease via the blood of bitten individuals. Owing to deforestation, insect vectors can now invade cities and are thus considered responsible for developing new endemic regions of disease. Many of the affected countries spend enormous resources to control insect vectors, usually entailing the use of chemical insecticides. However, chemical insecticides are considered to possibly cause side effects on humans and are detrimental to the environment. Other strategies have been developed including biological control and photo-insecticides.

Photo-activated insecticides were demonstrated to be a novel compelling alternative to chemical insecticides. The ideal photo-activated insecticides possess the following characters. First of all they are non-toxic in the dark and second they are not permanently accumulated in the environment, because they are usually bleached and chemically degraded by light. The third is that usually, they employ light energy to produce their phototoxicity and are intrinsically less harmful to the environment because they are used at very low concentrations. The concept of using PDT inactivation of pest populations was realized as early as in the 1930's using xanthene derivatives against *Anopheles* and *Aedes* larvae and the developments in this field have been reviewed by Ben Amor and Jori [164]. Among the limited reports in this area, porphyrins are particularly interesting as photo-insecticides

because they exhibit several absorption bands in the UV/visible spectrum, being therefore efficiently excited by the solar spectrum [165]. Under this concept, Lucantoni *et al.* tested the phototoxicity of a synthetic meso-substituted porphyrin meso-tri(N-methylpyridyl), meso-mono(N-tetradecylpyridil) porphine (C14) [166]. The photo-activable porphyrin showed phototoxicity on the dengue vector *Aedes aegypti* in laboratory conditions. In artificial pool tests, greatest population reductions were achieved using dosages on the countryside of 20 and 40 g/ha. Shao *et al.* synthesized a series of tetraethynyl silanes (TETS) as photo-activated insecticides to test their killing effects on 4th-instar larvae of *A. albopictus* (Skuse) [167]. After 3h incubation in a dark room, *A. albopictus* were treated by ultraviolet irradiation (300–400 nm, maximum at 365 nm) for 1.5h at the fluence rate of 2.074 wW/cm^2. Among them, the compound with four thiophene groups attached on the same silicon atom with the triple bond exhibited excellent photo-activated insecticidal activity. Relationship analysis between structure and activity showed that the presence of thiophene ring was critical for the overall activity [167].

3.8 Blood product disinfection by PDT

Transfusion-related infections and consequent fatalities continue to be reported in quite a few publications [168,169], and blood is currently not tested for many potentially dangerous known pathogens. In order to guarantee the safety of blood products from pathogenic microbes, PDT is an alternative approach with the main advantage of being potentially efficient against viruses, bacteria, fungi, and parasites. However, the main challenge in blood disinfection is to kill or inactivate the target microorganism without disruption of blood cells or serum proteins and moreover the PS should be without toxicity. MB is not an ideal PS for this application due to its ineffectiveness against intracellular viruses and tendency to cause collateral damage to blood components, typically to factor VIII and fibrinogen [170]. Thionin, the demethylated derivative of MB, mediating PDT has been shown to be efficient without damaging red blood cells and platelets [171]. Dimethylmethylene blue (DMMB), one type of phenothiazinium dye, illuminated by visible light showed good effects on the inactivation of DNA and RNA model viruses, including vesicular stomatitis virus and duck HBV (DHBV, a model for HBV; single-and double-stranded DNA virus), and leukocytes in RBCs. However, clinical studies have not yet been started with DMMB [172–174].

The naturally occurring vitamin B2, riboflavin (Fig 4G), illuminated by UVA or visible light, has also been developed as a nucleic acid-binding agent to be used for pathogen inactivation which results in photoinactivation of nucleic acid-containing pathogens in plasma, platelets, and RBCs. Preclinical studies have revealed the effectiveness of reduction in infectivity of some viruses, including HIV-1, bovine viral diarrhea virus (BVDV), a model for HCV; single-stranded RNA virus), and pseudorabies virus, as well as bacteria in blood products [175–178].

3.9 Aquaculture disinfection by PDT

Aquaculture comprises all types of culture of fish and other aquatic animals as well as culture of plants in fresh, brackish and marine environments. These enterprises are susceptible to water-borne disease caused by bacteria, viruses, helminths, protists and oomycetes, to a lesser extent, fungi. Among them, bacterial infection is one of the major concerns in the aquaculture industry [179]. Application of immobilized PS could mediate PDT to inactivate fish pathogenic microorganisms and if successful would manifests obvious advantage without low risk neither to the fishes nor to the environment. Only a few studies have been explored in this field, but preliminary results were gained at both laboratory level and at pilot stations. Porphyrin derivatives, as the PSs, have also a great potential for the disinfection of fish farming plant water, including Gram-positive bacteria

(e.g. MRSA), Gram-negative bacteria (e.g. *E. coli*), fungi (e.g. *C. albicans*) and fungal-like pathogens (e.g. *Saprolegnia spp.*) and parasitic protozoa (e.g. *Acanthamoeba palestinensis*) [179]. Microbial population was decreased 5–6 $\log_{10}$ CFU after 10 min of irradiation with a low fluence rate (ca. 50 mW/cm^2) in the presence of micromolar PS concentration [180]. Another study showed that a micromolar concentration of a porphyrinic PS eliminated the naturally or artificially induced infection of saprolegniosis in trout farming pools (inactivation of 6–7 $\log_{10}$) without collateral damage of the fish. The same low concentrations demonstrated also higher phototoxicity activity over more traditional pathogens MRSA and *E. coli* (up to 7 $\log_{10}$ decrease in bacterial CFU) [181]. The bactericidal effects of PDT were also exhibited for *V. vulnificus* that frequently infects fish farming water [182]. Similarly, cationic porphyrins-induced PDT inactivated up to 7 $\log_{10}$ of ten bacterial species (i.e. *V. anguilarum*, *V. parahaemolyticus*, *Photobacterium damselae subsp. piscicida*, *Photobacterium damselae subsp. damselae*, *E. coli*, *Enterobacter*, *A. salmonicida*, *S. aureus*, *Pseudomonas sp*, *E. faecalis*,) isolated from fish farming plant waters *in vitro* [179].

3.10 Waste water disinfection by PDT

At present, antimicrobial PDT is considered to have potential for use in the environmental area, including water disinfection. Effects were observed on the eradication of faecal bacteria [183–186], viruses [187] and helminth eggs [188] in environmental water. Bonnett *et al.* used a phthalocyanine immobilized on a polymeric membrane of chitosan in a model reactor of water disinfection [189]. Two mono-hydroxyl zinc-porphyrin derivatives with anionic and cationic net charge, respectively, were immobilized on poly(methyl methacrylate). The cationic derivative immobilized on poly(methyl methacrylate) polymer showed more effective inhibitory effect on the photo-inactivation of *Deinococcus radiodurans* [179].

3.11 Food disinfection by PDT

Numerous outbreaks of foodborne illness have been attributed to the contamination of produce due to inadequate sanitation of food contact surfaces [190]. Recent research has indicated that pathogens can acquire resistance to commonly used sanitizers or disinfectants and, as a result of such adaptation, cross-resistance of pathogens to antibiotics and production of multi-antibiotic resistant pathogens have been observed, which is a risk to animal health status and the safety of food products [191]. Brovko *et al.* investigated the photodynamic bactericidal effect of acriflavine neutral, phloxine B, RB and malachite green (oxalate salt) against two Gram-negative strains (*E. coli* LJH 128 and *Salmonella Typhimurium* C1058), two Gram-positive strains (*Bacillus sp.* C578 and *Listeria monocytogenes* LJH 375), and yeast (*Saccharomyces cerevisiae* C1172) [191]. The results showed that acriflavine neutral-induced PDT significantly reduced the viabilities of all pathogens tested. RB caused a significant reduction of bacterial viability incubated both in the dark and under illumination. Malachite green induced PDT was gave more effective inactivation of Gram-positive bacteria than Gram-negative bacteria and yeasts. Phloxine B demonstrated a significant photoinactivation on Gram-positive bacteria, whether in the dark or under illumination; but Gram-negative bacteria and yeasts were unaffected. Conjugation of RB and phloxine B with poly(vinylamine) resulted in an enhanced bactericidal effect during both dark and light incubation. No killing effect was observed for yeasts incubated with dye conjugates. The presented data suggest that a photodynamic approach for constructing "self-decontaminating" materials has potential in the food industry.

3.12 Effects of PDT on host cells and tissues

As mentioned above, PDT is an alternative approach to inactivate pathogenic microorganisms. The ideal outcome of antimicrobial PDT is that the target pathogenic

microbes are selectively inactivated while host cells and tissues are preserved. Therefore, one of the criteria to evaluate the effectiveness of a PS is that it should demonstrate selectivity of microbial cells over host tissue and cells [192]. A recent study indicates that using tetracationic phthalocyanine (RLP068) as a PS leads to an about 5 $\log_{10}$ decrease in the CFU of microbial pathogens with no collateral damage at the same doses to typical human cells, such as fibroblasts and keratinocytes [36]. Soukos *et al.* found that poly-L-lysine (pL)-chlorin e6 (ce6) conjugates could efficiently target photo-destruction towards Gram-negative (*P. gingivalis*) and Gram-positive (*Actinomyces viscosus*) oral bacterial species while it spared the oral epithelial cell line (HCPC-1) [193]. Tegos *et al.* investigated the broad-spectrum antimicrobial photodynamic activities induced by two series of functionalized C_{60}; one series had one, two, or three polar diserinol groups (BF1–3), another series had one, two, or three quarternary pyrrolidinium groups (BF4–6). The bis- and tris-cationic fullerenes showed highly germicidal effects on all tested microbes (4–6 $\log_{10}$), including Gram-positive bacteria, Gram-negative bacteria, and fungi, without any harm to mammalian cells (fibroblasts) [192]. One *in vivo* research, carried out by Trindade *et al.* evaluated the toxicity of Photogem® mediated PDT on rat palatal mucosa. Their data demonstrated Photogem®-induced PDT was not toxic to the rat palatal mucosa detected by macroscopic analysis and thermal mapping [194]. An *in vitro* study was carried out by Zeina *et al.* to investigate the effect of PDT on keratinocytes [195]. They found a therapeutic/ dosing protocol whereby PDT inactivated microorganisms effectively without damaging adjacent keratinocytes. The researchers used a combination of MB and visible light previously used for microbial killing. The kill rates for keratinocytes were 200-fold lower than for bacteria and 18-fold lower than for *Candida*. Using different PSs, Tanaka *et al.* demonstrated the cytocidal effects of PDT on neutrophils [196]. They studied the morphological alteration and the viability of murine peripheral-blood neutrophils after PDT performed *in vitro* by using each PS at a concentration that achieved a maximum germicidal effect on MRSA. Most neutrophils survived (>80%) after PDT using TBO or MB, however, other PSs used for PDT (such as erythrosine B, RB, Photofrin, crystal violet, NMB and Laserphyrin) caused morphological change and viabilities reduction (<70%) to neutrophils. Shrestha *et al.* evaluated the potential of a PS-conjugated chitosan as a matrix-reinforcing agent on dentin collagen [197]. Rose Bengal-conjugated chitosan (CSRB) was synthesized by using a chemical cross-linking technique and it was characterized for its photobiological, photophysical, and cytotoxicity properties. Dentin collagen was applied for the CSRB cross-linking experiments and later on evaluated for mechanical properties, chemical changes, and resistance to enzymatic degradation. The CSRB-cross-linked dentin collagen demonstrated higher resistance to collagenase degradation and superior mechanical properties.

In conclusion, *in vitro* researches have shown that, under the same dosimetric parameters of the photodynamic process, host cells are less sensitive to PDI than bacteria, suggesting a safety margin between bacterial inactivation and host cell damage if PDT is used *in vivo*. This selectivity might be due to the protection that the plasma and nuclear membrane afford in mammalian cells that may act as an additional barrier for the PS or the PS high-energy reaction products (ROS) formed after light. Differences in sensitivity may also reflect differences in cell volume/size which increase the amount of ROS needed to kill the cell. Mammalian cells are approximately 25–50 times larger than the bacterial test species and may therefore they are likely harder to be killed [195]. The selectivity can also be explained by the selective uptake of cationic PSs by bacteria compared to that by mammalian cells [193].

3.13 Clinical applications of anti-infective PDT

Although PDT was discovered in the antimicrobial field over 100 years ago, the first clinical trials in humans did not take place until 1970s. The clinical applications of anti-infective

PDT have been also evaluated and performed *in vivo*. Numerous studies indicate that PDT appears to be very efficient for the treatment of superficial and localized infections, including dental disease, cutaneous superficial and subcutaneous infection, gastric infections, and so on.

Qin *et al.* found an about 21% killing rate of microorganism in biofilms collected from patients with periodontitis, using 1 mg/ml TBO irradiated by 12 J/cm^2 red laser [198]. Dörtbudak *et al.* reported that TBO-PDT successfully decontaminated implants with bacterial colonization of 15 patients, reducing about 2log$_{10}$ bacterial CFU [199]. Soukos *et al.* used MB (25 μg/ml) PDT, illuminated by 665 nm red light (30 J/cm^2), to fully inactivate all bacterial species except *E. faecalis* (53% killed) in infected root canals of extracted teeth [200]. A 97% elimination of bacteria biofilm infected by *E. faecalis* in root canals were achieved by PDT with the same concentration of MB and increased fluence (222 J/cm^2) of red light. Fontana *et al.* reported that MB -PDT eliminated 63% subgingival species in the planktonic phase and 31% in the biofilm derived from patients' dental plaque with chronic periodontitis [201]. Another study, presented by William *et al.* showed that TBO-PDT could reduce 10-fold *S. mutans* which was present in a collagen matrix similar to mimicking carious dentin or appearing in decayed teeth [202]. Mima *et al.* used Photogem-PDT to treat five cases of denture stomatitis infected by *Candida spp.* The results showed four patients achieved clinical resolution and one patient demonstrated reduction in palatal inflammation. Two patients occurred recurrence of denture stomatitis during the follow-up period [203]. In one randomized clinical trial, Photogem®-induced PDT significantly reduced the CFU/ml of Candida species and showed clinical success rates 45% as effective as topical nystatin in the treatment of denture stomatitis [204].

Lee *et al.* used MAL-mediated PDT to treat six patients with refractory Malassezia folliculitis infected by *Malassezia* yeasts [205]. The results showed that a great improvement was achieved in four patients following continuous three sessions of PDT and a moderate improvement was achieved in one patient. Piracinini *et al.* treated a patient with recalcitrant onychomycosis infected by *T. rubrum* who did not respond to the topical and systemic antifungal agents' treatment for 18 months. Following three sessions of ALA-PDT in a 15-days interval, remission of the infection was released and without recurrence during a 24-months' follow-up period [206]. In another contrary study, thirty patients received ALA-PDT and achieved a 43.3% cure rate 12 months after treatment, however, cure rate dropped down to 36.6% after 18 months [207]. Nine patients with interdigital mycoses of the feet received 20% ALA-PDT treatment and achieved good therapeutic effects in most patients, however recurrences were seen quickly [208].

Another compelling application of PDT for superficial cutaneous infection is the treatment of acne vulgaris. Hongcharu *et al.* first revealed the efficacy of topical ALA-PDT to treat acne vulgaris on the back in a small but well administrated study in adults [209]. Another study was carried out by Itoh *et al.* using topical 20% ALA irradiated by lower fluence red light (13J/cm^2) in Japanese patients. The acne lesions were improved and lasted at least 6 months [210,211]. Gold *et al.* evaluated the efficacy of ALA-PDT illuminated by intense pulsed light (IPL) and other light sources on moderate to severe inflammatory acne vulgaris in a clinical trial. Twenty patients were enrolled. Among them, fifteen completed the whole trial and twelve patients responded to the treatment. Among respondents, 50.1% patients reduced the active inflammatory acne lesions at the end of the four-week treatment, 68.5% was improved four weeks after the last treatment, and 71.8% was improved twelve weeks after the last treatment [212]. A large sample, randomized, split-face, placebo-controlled study was carried out by Yin *et al.* in China in 2010 [213]. These authors recruited one hundred and eighty patients and compared different topical ALA concentrations vs. placebo for PDT in patients with moderate to severe acne. Every patient was treated once at intervals

of 10-days for four sessions. After 24 weeks, the sides treated by ALA-PDT demonstrated clinical improvement compared with the control sides treated only by red light ($P < 0.01$) (Fig 5). The beneficial outcomes were dose-dependent; however, the authors pointed out that the occurrence of side-effects also showed a dose-dependent increase.

There have been further studies reporting beneficial effects of PDT on various bacterial infections, acne vulgaris, leishmaniasis, viral infections, gastric infections, etc. Table 2 summarizes the representative clinical applications of antimicrobial PDT. Although the overall clinical outcomes in all the studies were considered to be positive, discrepancies were found in the therapeutic efficacies in different trials. It was likely based on the use of different light sources, different PSs, concentration and incubation time between PS and infected tissue.

By and large, PDT has been investigated in the laboratory and the clinic, and was found to be able to inactivate numerous pathogenic microorganisms efficiently. However, the application of PDT as an anti-infective in the clinic is still at a relatively early stage and faces some limitations that need to be overcome before it can assume its future application as a therapeutic approach for deep infections. PDT will not replace antimicrobial chemotherapy, but may improve the treatment of localized infections, promote healing and reduce the cost of the treatment with low side-effects. It would be prudent to say that there is not any convincing evidence to support s that PDT has superior advantages over the traditional modalities at present. Furthermore, meta-analyses and randomized long term clinical studies are necessary to confirm the beneficial effect of antimicrobial PDT according to the tenets of evidence-based medicine. Further studies on the exploration of new PSs and more efficient light delivery systems are required to establish optimal treatment parameters. Antimicrobial PDT may hold promise as a substitute for currently available antibiotic therapy in the treatment of recalcitrant, recurrent, and multi-drug resistant bacterial and fungal infections especially in poorly vascularized tissues where antibiotics have difficulty reaching.

4 Blue light for infectious disease

4.1 Introduction

Another novel light-based approach, the use of blue light (400–500nm) as a mono-therapy, is gaining increasing attention owing to its potential antimicrobial effect without addition of exogenous PS. Moreover, it is accepted that blue light is much less harmful to mammalian cells than ultraviolet irradiation [214]. In addition to safety benefits, prolonged exposure of materials to 405-nm visible light would not induce the problematic levels of photodegradation that are associated with similar periods of material exposure to UV light, particularly in the UV-C region of around 254 to 260 nm. The biological response of blue light was firstly reported in 1881 by Darwin who reported phototropic response in plants to blue light. Up to now, the phototropic response of blue light has been identified in all three biological domains, including bacteria, eukaryotes, and archaea [215]. Recently, new exciting discoveries have been made concerning the genomes of many photosynthetic and chemotropic bacteria and fungi, which can encode photoreceptor proteins. For examples, blue light receptors have been studied in *Neurospora crassa* and other fungi, which can be divided into two general classes: the cryptochromes and the phototropins. The two major blue light photoreceptors are, (i) white collar (WC)-1 and (WC)-2, which control dark to light transition and (ii) VIVID, a protein important in the second light signaling system. Together, they control responses to daily changes in light intensity and also are involved in the modulation of the circadian clock [216,217]. Other photoreceptors have been studied in *E.coli* [218] and *A. baumannii* [219] and are functional coded for production of a BLUF protein (blue light sensing using flavin), encoded by the blue-light-sensing A (bslA) gene.

The discoveries of those photoreceptors have triggered the new concept that not only phototrophic bacteria but also photosynthetic and chemotrophic microorganisms can sense light [220]. So far, it has been found that some bacteria and fungi can chose their lifestyle between the motile single-cellular planktonic state and the multicellular surface-attached community state (biofilms) by photo-regulated pathways, including the second messenger cyclic di-GMP system, and direct interactions of photoreceptors with transcription factors [221,222]. Blue light has been verified as a crucial factor that can affect all these physiological responses and decide the bacterial lifestyle [223,224]. Blue light not only can regulate bacterial motility, suppress biofilm formation, but also subsequently potentiate light inactivation of bacteria. On the other hand, the presence of blue light may also activate or increase bacterial virulence [219].

The lethality of blue light for bacteria has been demonstrated both *in vitro* and *in vivo* which can produce a broad-spectrum bactericidal effect on both Gram-negative and Gram-positive bacteria. While the wavelength range of 402–420 nm has been reported to be the most effective antimicrobial spectral range, both 455 nm and 470 nm wavelengths have also been found to be of antimicrobial potential for some bacterial species (e.g., *S. aureus*) [219]. The precise mechanism of the antimicrobial effect of blue light is not fully elaborated yet. The generally accepted hypothesis is that blue light excites endogenous intracellular porphyrins to behave as PSs, and this photon absorption sequentially leads to energy transfer and ultimately, the production of highly cytotoxic ROS – most notably $^1O_2$ in a similar manner to PDT [225]. However, different bacteria demonstrate variable susceptibilities to blue light. Studies have reported that Gram-positive species, in general, were more sensitive to 405 nm light inactivation than Gram-negative species, which is generally consistent with the results obtained in a recent study [226,227]. It has also been theorized that the differences in inactivation kinetics may be due to organism-specific differences in porphyrin levels, individual porphyrin sub-types (*i.e.*, porphyrin wavelength absorption maxima), or as a consequence of the relatively short distance that $^1O_2$ molecules can diffuse (~20 nm in solution) within cell structures [33,226]. In addition, it has been speculated that less oxygen-tolerant bacterial species may be particularly susceptible to the effects of ROS as microorganisms, such as some microaerophilic species, have been found to possess fewer key antioxidant defenses than most aerobes [228,229]. For example, studies have demonstrated that blue light is able to inactivate the anaerobic oral pathogens *Prevotella*, *Porphyromonas*, and *Fusobacterium* as well as microaerophilic pathogens such as *P. acnes* and *H. pylori* [33,230]. However, inactivation results achieved with anaerobic/microaerophilic bacteria have not provided conclusive evidence as to whether oxygen-sensitive anaerobic bacteria are any more susceptible to blue light than aerobes as many of these bacteria are also known to accumulate high levels of porphyrins. Possible explanations for this difference in inactivation susceptibility could be that different characteristics of bacteria, the unusual cell envelope, confers some resistance to 405 nm light penetration and/or that bacterial cell envelopes provide greater innate resistance to ROS given that bacteria species are capable of evading phagocytic oxidative burst damage [227].

4.2 Blue light inactivation of bacteria and fungi

Blue light is lethal to many species of bacteria and fungi, even including filamentous fungi, although the effectiveness is dependent on the light wavelength, energy levels and microbial genus and species [216]. Blue non-coherent light sources, such as LED, the halogen lamp, and the plasma-arc curing (PAC) light, are often used *in vivo* and *in vitro* studies. The bactericidal efficacy *in vitro* of blue light for *Escherichia coli*, MRSA and *P. aeruginosa*, even *Streptococcus mutans* in biofilms has been reported [231].

Maclean *et al.* studied the bactericidal effect of 405-nm blue light emitted from a LED array on some bacterial species, including Gram-positive *S. epidermidis*, *S. aureus, Enterococcus*

faecalis, Streptococcus pyogenes, Clostridium perfringens, Gram-negative *E. coli, Acinetobacter baumannii, P. aeruginosa, Proteus vulgaris* and *K. pneumonia* which are all associated with hospital acquired infection [226]. The results demonstrated that most of Gram-positive and Gram-negative species were successfully eliminated, except *E. faecalis* which was the least sensitive to inactivation by 405-nm blue light.

Murdoch *et al.* reported that bactericidal effect of high-intensity 405nm light, delivered by an array of LED, on taxonomically diverse bacterial pathogens from the genera *Salmonella, Shigella, Escherichia, Listeria, and Mycobacterium* [227]. *L. monocytogenes* was most readily inactivated in suspension, whereas *S. enterica* was most resistant. In surface exposure tests, *L. monocytogenes* was more susceptible than Gram-negative enteric bacteria to 405nm light when exposed on an agar surface but interestingly less susceptible than *S. enterica* after drying onto PVC and acrylic surfaces. *Mycobacterium terrae,* which was chosen as a safe, comparative, surrogate microorganism for the highly pathogenic *M. tuberculosis,* was inactivated by 4–5 $\log_{10}$ CFU/ml between a dose of 144 and 288 J/cm^2 by 405nm light.

Blue light photoinactivation—as evaluated for other bacteria such as *Helicobacter pylori, Propionibacterium acnes, Porphyromonas gingivalis* and some black-pigmented bacteria [225,232–234] —has been attributed to the photo-excitation of endogenous intracellular porphyrins by blue light in the wavelength region from 400 nm to 500 nm and, more particularly, 400 nm to 420 nm in the cases of *H. pylori* and *P. acnes* [225,232]. The studies on the *in vitro* antimicrobial effect of blue light are summarized in Table 3

However, the types and the amount of porphyrins can lead to different photo-inactivation rates in different bacteria. For example, Gram-positive *S. epidermidis* and *S. aureus* produced coproporphyrin as the predominant porphyrin species, and these species produce 2–3 times higher coproporphyrin than in Gram-negative strains (*i.e. E. coli, Aeromonas* strains and *Acinetobacter*) whereas there was no primary porphyrin produced in some Gram-negative bacteria [235]. Owing to fact that different bacteria produce different predominant porphyrins and their peak absorption wavelengths of these porphyrins may be at variance. As a consequence, different wavelengths may be required for their optimal photo-stimulation. All the aforementioned factors clearly induced the diversity of bactericidal efficacy by blue light. Other factor that must be taken into account are that the bacteria may produce different levels of porphyrins when cultured in laboratory cultivation conditions than they do in their natural physiological states, such as when they are infecting a mammalian host [236].

Fungi are able to sense light in a broad spectrum range, from UV to far-red light because they have blue-light receptors. Flavins or flavoproteins are considered to act as blue light receptors and can absorb blue light [237]. This observation makes them able to serve as a cue' in the asexual development of fungal spores, and affecting growth, metabolism, pigment formation, and tropism [238,239]. This observation makes them able to serve as a cue' in the asexual development of fungal spores, and affecting growth, metabolism, pigment formation, and tropism [237], or could photoexcite endogenous porphyrins in a similar manner to PDT [240]. However, there have been few reports demonstrating the fungicidal efficacy of blue light. Only recently, De Lucca *et al.* found that blue light (470nm) alone significantly reduced *Fusarium graminearum* (FG) germinating conidial viability by approximately 36, 42, and 47% respectively at fluences of 40, 80 and 100 J/cm^2 [216]. However, no significant viability loss was observed for the FG non-germinated conidia treated with blue light (20–100 J/cm^2).

4.3 Effects of blue light on host cells and tissues

In order to use blue light for the treatment of infectious diseases, it is very important to assess the possible harm that the blue light could cause against host cells and tissues so that they can avoid non-specific damage. However, only a few studies have been conducted so far in this area.

In one *in vitro* study, Hockberguer *et al.* showed that some cells, such as mouse 3T3 fibroblasts, foreskin keratinocytes from human, kidney epithelial cells from monkey were irradiated with blue light (450nm at 6.3 W/cm^2) for 20 min respectively, produced hydrogen peroxide (H_2O_2), an important ROS which can lead cellular damage and could cause pathological changes associated with exposure to blue light [241].

Wataha *et al.* treated 3T3 mouse fibroblasts with three dental blue (400nm–500nm) light sources, and detected succinate dehydrogenase (SDH) activity of mitochondria after a range of fluences (1.3 to 60 J/cm^2) [242]. Results showed that fluences ranging from 5 J/cm^2 to 15 J/cm^2 irreversibly completely suppressed SDH activity compared to dark controls up to 72 h post-exposure.

Godley *et al.* investigated the interaction of human primary retinal epithelial (PRE) cells with blue light (390–550 nm at 2.8 mW/cm^2) and reported significant damage to the mitochondrial DNA after 3 h of light exposure. At the same time, release of ROS ($^1O_2$, $O_2^-\bullet$, HO$\bullet$) increased in isolated mitochondria after irradiation. Hence, it has been confirmed that blue light can damage cells via ROS release and subsequent DNA damage [243].

Shnitkind *et al.* studied the effects of blue light illumination on two immortalized keratinocyte cell lines, HaCaT and hTERT1. The cells were irradiated by 420nm blue light at fluences of 54 or 134 mJ/cm^2. The results showed that blue light inhibited IL-1 release, which proved that blue light has anti-inflammatory potential on keratinocytes [244].

Conversely, a recent clinical research performed by Kleinpenning *et al.* did not demonstrate significant side effects on human skin [214]. Eight healthy volunteers were illuminated with a blue light source (390–460 nm) with a peak emission of 420 nm over 5 consecutive days at 20 J/cm^2. To analyze the irradiation effects, skin biopsies were analyzed for p53 expression, vacuolization, sunburn cells, elastosis, matrix metalloproteinase-1 (MMP-1), and melan-A expression. As a result, neither inflammatory nor sunburn cells were observed before or after irradiation. No significant changes in p53 and MMP-1 expressions or sign of elastosis were observed after blue light exposure. Keratinocytes demonstrated a significant incremental perinuclear vacuolization during treatment (P=0.02) with a tendency towards significance after cessation of treatment (P=0.09). Irradiated skin demonstrated minor hyperpigmentation and a significant increase in melan-A-positive cells (P=0.03) histologically. Therefore, the investigators concluded that short-term use of blue light in cutaneous treatment could be considered safe.

In conclusion, blue light has been occasionally reported to be phototoxic to mammalian cells at certain spectrum ranges in a wavelength dependent manner. The photocytotoxic effect was assumed to be similar to that which occurred in bacteria, such as photo-excitation of intracellular chromophores and subsequent cytotoxic ROS formation. Thus, an ideal blue light therapy should choose an optimal wavelength to selectively excite the chromophores in pathogenic bacteria with minimized effect on chromophores in mammalian cells [219].

4.4 The clinical application of blue light for infectious diseases

One of the major application of blue light for infectious disease in the clinic is the treatment on acne vulgaris which is an important dermatologic disorder infected mainly by *P. acnes*. Several studies have reported the benefits of blue light on acne vulgaris. Table 4 summarizes the clinical studies of blue light therapy for acne vulgaris.

Blue light also can inactivate *H. pylori*, which is the primary pathogen causing atrophic gastritis and peptic ulcers and is increasingly resistant to antibiotics [245]. Ganz *et al.* treated 10 patients with blue light (405 nm, 40.5J/cm^2) via an optical fiber inserted in the endoscope channel that illuminated a 1-cm diameter area of the gastric mucosa [246]. Significant decreases (91%) in *H. pylori* colonies per gram of tissue in treated sites was observed by comparing biopsies from treated and adjacent untreated sites. Some patients showed reductions of bacterial burden approaching 99%. Another pilot trial was carried out by Lembo *et al.* using a blue (408 nm) laser source with multi-segmented balloon that allowed whole stomach illumination to treat 18 patients with *H. pylori* infection [247]. A generalized reduction in bacterial load was observed in the stomach. Significant reduction in bacterial colonization was found in the antrum (>97%), followed by the body (>95%) and the fundus (>86%) with no apparent dose-response correlation. However, repeated breath tests showed *H. pylori* repopulated in days following illumination.

In conclusion, blue light can treat inflammatory and non-inflammatory acne lesions caused by *P. acnes* and digestive disorders caused by *H. pylori*. Blue light therapy has been demonstrated in clinical studies to be feasible and safe, and may represent a novel alternative approach to the eradication of *P. acnes* and *H. pylori*, especially in patients who have failed standard antibiotic treatment. However further studies are necessary to establish appropriate protocols for each clinical condition.

4.5 Summary

In summary, blue light, at certain wavelengths (range 402–420 nm), has been found to be effective in the inactivation of pathogens in both laboratory and clinic. The overall outcomes were demonstrated to be positive in all the studies. This is a new compelling approach that can eliminate a wide range of medically important pathogenic bacteria, including MRSA, *P. aeruginosa*, *A. baumannii* and *E. faecalis*. The absence of a requirement for pretreatment with a PS increases its likelihood for widespread applications, although discrepancies have been seen in the antibacterial efficacy in different studies. It was likely that the differences were owing to the different light delivery regimen used in the studies, differences in the spectra of light sources, differences in light fluences, variable microbial genera, different patient populations, *etc*. In common, the clinical application of blue light for infectious diseases is still at the early stage of development. The sample sizes of all published clinical studies are still very limited and the target bacteria are mainly *P. acnes* and *H. pylori*. Further studies with larger sample sizes are warranted to optimize the parameters of blue light therapy, and to investigate the use of blue light for infections caused by a wider range of pathogens. Further development of blue light inactivation approaches could lead to potential applications as decontamination systems for air, contact surfaces, and medical instruments within the clinical environment [248,249]. Light with wavelengths centered at 405-nm has shown a significantly broad antibacterial spectrum which offer potential application in numerous fields including food sterilization, prevention of contamination, sewage disposal and other disinfection industries [236].

5. Other light-based anti-infectives

5.1. Anti-infective effect of femtosecond pulsed lasers

It has been proposed that femtosecond lasers, or lasers that have a pulse duration in the region 10^{-15}s, are able to break down transparent or semitransparent biological tissues due to nonlinear absorption of laser energy with minimal thermal and mechanical effects [250]. As a result of the adverse thermal damage possible with other laser systems, femtosecond lasers have been hypothesized to be a new approach as anti-infectives. The basic idea is that the entire energy of a pulse (which could be quite small) is delivered in a very short time, so if the average power is 10 mW and the pulse duration is 100 fs then the peak power is 100 GW which is sufficient to produce efficient 2-photon absorption. Recently, a series of studies have demonstrated the efficacy of a visible femtosecond laser or a near-infrared subpicosecond fiber laser on elimination of several kinds of viral species, including *M13 bacteriophage*, *tobacco mosaic virus*, HPV, and *human immunodeficiency virus* (HIV) [251–258]

In 2007, Tsen *et al.* found that a very low power visible femtosecond laser (as low as 0.5 nJ/ pulse, 425 nm wavelength, 100 fs pulse width, peak power density greater than or equal to 50 MW/cm^2) irradiated M13 phages efficiently [254]. Their further research demonstrated that one kind of visible femtosecond diode-pumped continuous-wave (CW) mode-locked Ti-sapphire laser (= 425 nm) could inactivate M13 bacteriophage, encephalomyocarditis virus and *Salmonella typhimurium.* The laser emitted a continuous array of 60 fs pulses at a repetition rate of 80 MHz, average power of about 50 mW. It has a pulse width of full-width at half maximum (FWHM) 100fs. The laser inactivated nearly the entire aforementioned microorganism very efficiently, especial for *Salmonella typhimurium* [258]. Another recent study reported the inactivation of murine cytomegalovirus (MCMV), an envelope, double-stranded DNA virus, illuminated by visible femtosecond laser (= 425 nm) which was reduced 5 log $_{10}$ titer and the virus demonstrated selective aggregation of viral capsid and tegument proteins [259]. However, the global structure of MCMV virions including membrane and capsid, was not changed significantly evaluated by electron microscopy; meanwhile the double-chain of MCMV genomic DNA did not show any break or crosslinking.

Manevitch and colleagues showed that the application of the femtosecond laser at a fluence of 7×10^{31} photons m^{-2} s^{-1} effectively and selectively inactivated *T. rubrum* from nail clippings obtained from infected toenails [260]. The laser wavelength used was 800nm which wavelength could effectively traverse the nail to eliminate dermatophytes at any level. Most importantly, it killed fungal cells without any collateral damage to the nail structure as confirmed by canning electron microscopy. This is the only study assessing the fungicidal efficacy of this laser system for onychomycosis *in vitro* at present. In fact, it is difficult to selectively delivery laser energy to microorganisms embedded in the nail plate without causing collateral damage to the nail structure overlying the fungi. Femtosecond infrared titanium sapphire lasers can theoretically circumvent this problem owing to the nonlinear interactions of femtosecond lasers with biological media and the ability to selectively deliver energy at the laser focus [261]. This study further demonstrated femtosecond infrared laser was a new alternative approach for the infection of fungus.

It has been proposed that use of a near-infrared (NIR) femtosecond laser via impulsive stimulated raman scattering (ISRS) to produce damage (e.g. to the protein coat of a virus) is a method for selectively inactivating microorganisms [254]. There were different mechanisms of inactivation of different microorganism by femtosecond laser. Inactivation of viruses involving the breaking of hydrogen/hydrophobic bonds or the separation of the weak protein links in the protein shell of a viral particle. On the contrary, inactivation of bacteria

is related to the damage of their DNA due to 2-photon absorption of a visible femtosecond laser [258].

When a NIR femtosecond laser induced the inaction of virus and bacteria, its safety to the mammalian cells was considered. The relative research demonstrated that if the wavelength and pulse width of the femtosecond laser were appropriately selected, there was a window in power density that enabled them to achieve selective inactivation of target viruses and bacteria without causing cytotoxicity to mammalian cells. It was suggested that this strategy targeted the mechanical (vibrational) properties of micro-organisms, and thus its antimicrobial efficacy was likely to be affected by genetic differences in the micro-organisms [255].

Advantages of such a new laser technology over the presently prevailed disinfection methods have been proposed to be: 1. it is a noninvasive disinfection technology, because no extraneous materials are needed in the disinfection process; 2.it is a harmless environmental disinfection method since no chemicals are used in the pathogen inactivation process; 3. it is a general method for selective disinfection of pathogens with potential minimal side effects [258].

5.2. Anti-infective effect of dual wavelength (870/930 nm) laser

Recently by using the wavelengths that had previously demonstrated cellular light mediated damage effects in optical traps, Bornstein *et al.* developed a dual wavelength laser system (with the wavelengths of 870 nm and 930 nm) for the inactivation of bacteria and fungi [262]. During *in vitro* studies, by using 1.5cm diameter flat-top light spots (fluence rate of 5.66 W/cm^2, at physiological temperatures), the investigators successfully inactivated *S. aureus, E. coli, C. albicans and T. rubrum.* Using non-lethal light fluence, the authors observed a decrease in transmembrane potentials and an increase in ROS generation in pathogenic microorganisms (MRSA and *C. albicans*) and human embryonic kidney cells. It was also postulated that the dual wavelengths caused an optically mediated — mechanotransduction of cellular redox pathways, decreased transmembrane potentials, and increased ROS generation. The cellular energetics of prokaryotic, fungal pathogens, as well as host cells, was affected in a similar manner when treated with the dual wavelengths at the fluence used. After thermal tolerance experiments in live porcine skin, human pilot studies were performed, examining the photo-inactivation of MRSA in the nose, and of fungi present in onychomycosis. The investigators did not observe non-specific damages either to the nares or the nail matrix, yet found successful photo-inactivation of pathogens.

Krespi *et al.* performed a prospective clinical study using a dual wavelength laser (870/930 nm) for nasal decolonization of *S. aureus* and MRSA [263]. Twenty-five patients colonized with *S. aureus* or MRSA were enrolled and non-randomly divided into 4 treatment groups: dual-wavelength laser with low-power alone (GR1); dual wavelength laser with low-power in combination with erythromycin (E-mycin) cream (GR2); dual wavelength laser with low-power in combination with peroxide irrigation (GR3); and single wavelength 940-nm laser with high-power alone (GR4). Laser illumination was delivered by using a fiber diffuser, with the fluences of 200 to 600 J/cm^2 for each naris circumferentially. Nasal decolonization efficacy for the groups of GR1, GR2, GR3, and GR4 was respectively 1 out of 3, 13 out of 14, 2 out of 4, and 4 out of 4. No side effects or discomfort were observed. The authors concluded that dual wavelength laser therapy could eradicate *S. aureus* and MRSA. It also could potentially re-sensitize bacteria which was drug-resistant to the common antibiotic, erythromycin.

A possible mechanism of the resensitization of bacteria to antibiotics using dual wavelength laser was that, the laser illumination interacted with the respiratory processes of the bacterial

plasma membranes, lowered the membrane potential, and subsequently inactivated the bacterial resistance mechanism, which depended on the membrane drug efflux pump [263].

6. Can pathogenic microorganisms develop resistance to light based anti-infectives?

Before light-based antimicrobials become widely used, one question that must be addressed is: can pathogenic microorganisms develop resistance to light-based anti-infectives? If this is indeed possible, the mechanism of resistance development is likely to be different for each light-based modality.

6.1 Can pathogenic microorganisms develop resistance to PDT?

An early study investigating the development of resistance by bacteria after PDI was carried out by Lauro *et al.* [264]. *Peptostreptococcus micros* and *Actinobacillus actinomycetemcomitans* were subject to 10 cycles of repeated sub-lethal PDI and regrowth using visible light combined with the PS consisting of porphycene-polylysine conjugates (2,7,12,17-tetrakis(2-methoxyethyl)-9-glutaramidoporphycene and 2,7,12,17-tetrakis(2-methoxyethyl)-9-p-carboxybenzyloxyporphycene). The investigators found that the repeated PDI of *P. micros* and *A. actinomycetemcomitans* by both PSs did not induce the development of resistance by bacteria to PDI in partially photo-inactivated bacteria.

Taveres *et al.* assessed the development of bacterial resistance to PDI mediated by white light and Tri-Py⁺-Me-PF (5,10,15-tris(1-Methylpyridinium-4-yl)-20-(pentafluorophenyl)-porphyrin triiodide) [265] *Vibrio fischeri* and recombinant *E. coli* were the studied bacteria. The results showed that after 10 consecutive cycles of sub-lethal PDI followed by regrowth, *E. coli* did not develop appreciable resistance to PDI. In another study, Giuliani *et al.* investigated the potential of *P. aeruginosa, S. aureus* and *C. albicans* strains to develop resistance to PDI after beingexposed to RLP068/Cl, a tetracationic Zn(II) phthalocyanine derivative [266]. The authors reported that 20 consecutive cycles of sub-lethal PDI with RLP068/Cl did not generate any resistant mutants.

6.2 Can pathogenic microorganisms develop resistance to UVC irradiation?

Dai *et al.* investigated the potential development of resistance to UVC (254 nm) inactivation by *T. rubrum* by performing five consecutive cycles of sub-lethal UVC inactivation followed by fungal regrowth *in vitro* [9]. The investigators did not observe significant difference in fungal inactivation rate among the five cycles of fungal inactivation and regrowth when 120 mJ/cm^2 UVC energy was delivered. The investigators concluded that the development of resistance to UVC inactivation is not rapidly acquired by *T. rubrum* that are subject to repeated sub-lethal UVC inactivation.

Alcantara-Diaz *et al.* evaluated the divergent adaptation of *E. coli* to repeated cycles of sub-lethal UVC inactivation at 254 nm [267]. Five cultures of wild type *E. coli* PQ30 were exposed to 80 consecutive bacterial inactivation by UVC and regrowth. The initial fluence of UVC was 1mJ/cm^2 for each cycle, and the fluence was increased 2-fold every 10 inactivation-regrowth cycles. Quantitative dose response curves were obtained by exposing the bacteria in exponentially growing phase to several UVC fluences. The investigators reported that all cultures were observed to develop different degrees resistance to UVC inactivation after 80 consecutive cycles of sub-lethal bacterial inactivation and regrowth. The adaptation of bacteria to cyclic UVC inactivation was believed to be a consequence of selecting advantageous mutations in those genes related to DNA repair and replication.

6.3 Can pathogenic microorganisms develop resistance to blue light?

To the best of our knowledge, this question has not yet been experimentally addressed. As the mechanism of blue light inactivation of microorganisms is suggested to be similar to that of PDI [219,225,268], it is expected that the potential of resistance development by pathogenic microorganisms to blue light inactivation is less than that of antibiotics. In a recent study carried out in our lab (data not published), we found that, after 10 consecutive cycles of sub-lethal bacterial (*A. baumannii*) inactivation by blue light at 415 nm and bacterial regrowth, there was a significant decrease (rather than an increase) in bacterial resistance to blue light inactivation between the first cycle and the 10th cycle (P <0.05). However, a contradictory finding was reported by Guffey *et al.* [261]. In that study, the investigators found that the inactivation of *S. aureus* by blue light (405 nm) was increasingly effective through the first 4 cycles of sub-lethal inactivation-regrowth (P<0.05). From the 5th cycle, the increase of bacterial resistance to blue light inactivation was observed (P<0.05).

6.4 Summary

There have been no reported cases of PDT induced resistance of microorganisms despite numerous attempts to induce resistance by repeated cycles of sub-lethal PDI and microbial re-growth. It is believed that PDT acts at multiple sites within microorganism cells [269], and, subsequently, offers less potential for the development of resistance by microorganisms to PDI than antibiotics, which usually act on a single-target. Development of resistance by microorganisms to UVC inactivation is possible after excessive repetition of UVC inactivation. This is because one primary function of UVC inactivation is to damage microbial DNA. However, for treatment of wound or other localized infections using UVC, only a limited number of repetitions of therapy are expected to be required. Variable results have been reported regarding the development of resistance by microorganisms to blue light inactivation. Further studies are warranted to elucidate the intrinsic mechanism of blue light inactivation of microorganisms.

7. Conclusion

Light based anti-infective therapies have become a rapidly growing area of interest in recent years. The reasons for this rise in interest are manifold; multi antibiotic resistance is the predominant growth-driving factor, but other relevant considerations may be the public distrust of big-pharmaceutical companies and their products, the desire to use —natural therapies and blue light is considered somewhat similar to sunlight; and the rapid microbial killing typically seen in phototherapy; so when the light is switched off the killing is done. Since many of the applications of phototherapy can in fact be performed by midday sunlight that has been suitable concentrated and/or filtered by cheap disposable devices, antimicrobial phototherapy may in future be transitioned into a therapy suitable for the developing world, where it is accepted that infectious diseases represent a much bigger burden to public health than they do in developed nations. As always in anti-infective therapies, selectivity towards microbial cells compared to host mammalian cells is also critical. Fortunately this appears to be the rule rather than exception for the phototherapies discussed above.

Acknowledgments

Abbreviations

ALA	5-aminolevulinic acid methyl ester
BCG	*M. bovis* Bacille Calmette Guerin
bFGF	basic fibroblast growth factor
BVDV	bovine viral diarrhea virus
CFU	colony forming units
ClAlPC	chloroaluminum-phthalocyanine
CPD	cyclobutane pyrimidine dimer
CSRB	chitosan-conjugated rose bengal
DMMB	dimethylmethylene blue
DP-mme	deuteroporphyrin mono-methylester
EDTA	ethyelenediamine tetraacetic acid
ER	erythrosine
GaPc	cationic gallium phthalocyanine
HA(B)	hypocrellin A(B)
Hp	hematoporphyrin
HPV	human papillomavirus
LED	light-emitting diode
MAL	5-aminolevulinic acid
MB	methylene blue
MDR	multi-drug resistant
MRSA	methicillin resistant *Staphylococcus aureus*
MSSA	methicillin sensitive *Staphylococcus aureus*
PDI	photodynamic inactivation
PDR	pan-drug resistant
PDT	photodynamic therapy
PEI-ce6	polyethylenimine chlorin(e6) conjugate
PS	photosensitizer
RB	rose Bengal
RBC	red blood cell
ROS	reactive oxygen species
TB	*Mycobacterium tuberculosis*
TBO	toluidine blue O
THPTS	meso-tetrahydroporphyrin-tetratosylate
TMP	tetra-substituted N-methyl-pyridylporphine
Tri-P(4)	5-phenyl-10,15,20-tris(N-methyl-4pyridyl)-porphine trichloride

UVC	ultraviolet c
VRE	vancomycin resistant *Enterococcus*
VSV	vesicular stomatitis virus
WGA	wheat germ agglutinin
XDR	extensively-drug resistant

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Highlights (for review)

Light-based anti-infectives work against biofilms and localized infections.

Ultraviolet C light will kill all pathogens without killing host mammalian cells.

Photodynamic inactivation produces reactive oxygen species that kill pathogens.

Blue light can kill many pathogens due to accumulation of porphyrins.

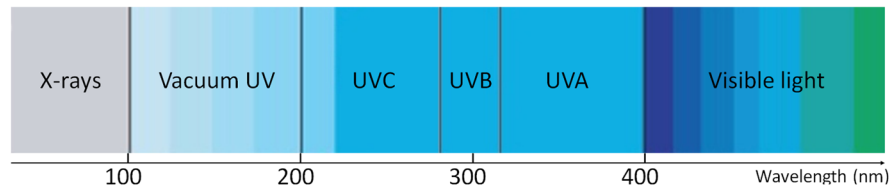Femtosecond infrared laser pulses can selectively kill pathogens.

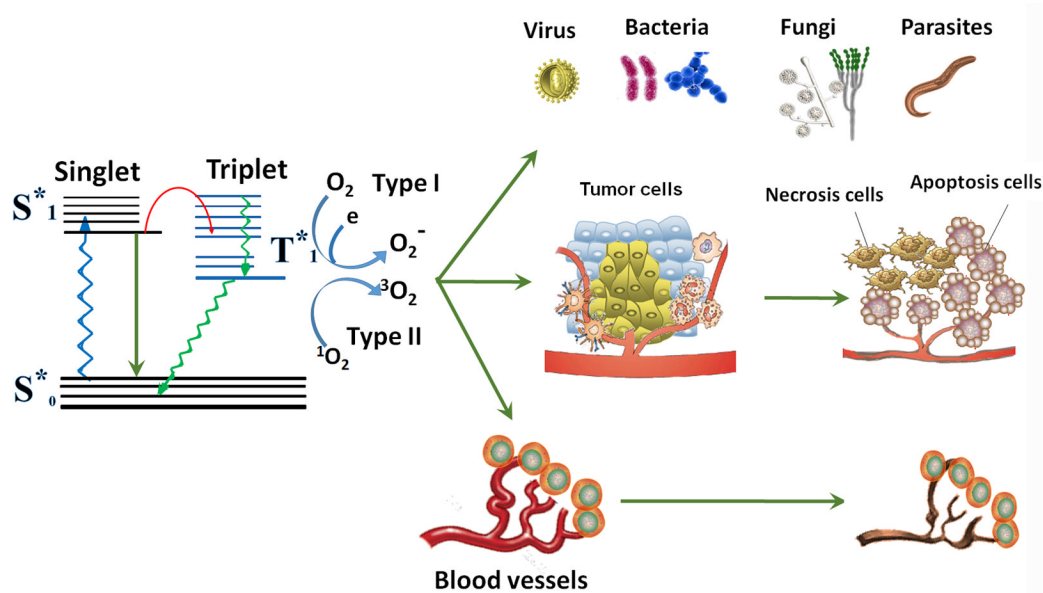**Figure 1.**
Spectrum of ultraviolet irradiation.

Figure 2.
Schematic illustration of photodynamic action. The PS initially absorbs a photon that excites it to the first excited singlet state and this can relax to the more long-lived triplet state. The triplet PS can interact with molecular oxygen in two pathways, Type I and Type II, leading to the formation of reactive oxygen species (ROS) and singlet oxygen $^1O_2$, respectively. The formation of ROS and $^1O_2$ can chemically attack a very wide range of biomolecules.
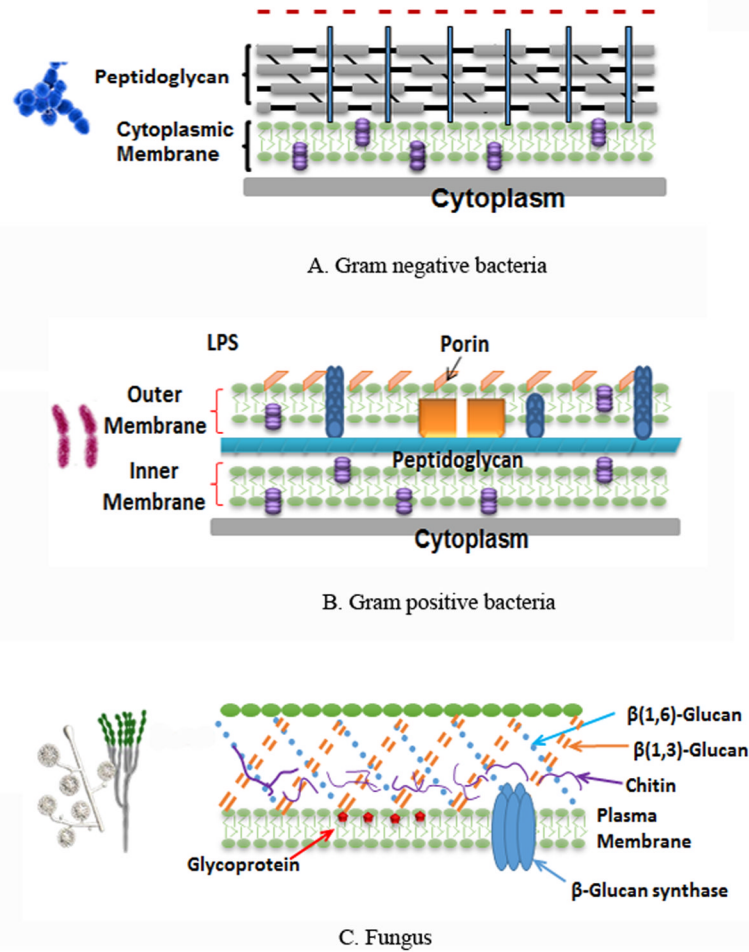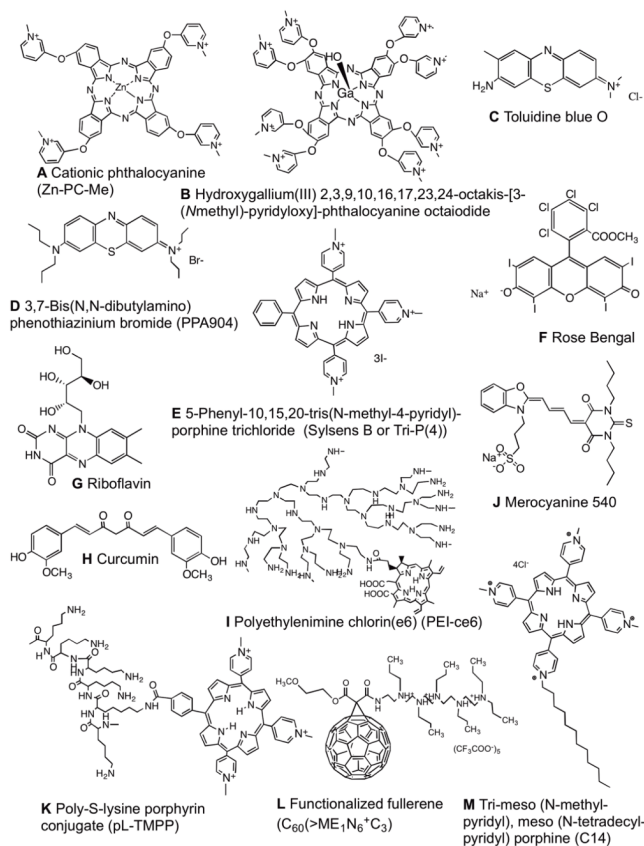
Figure 3.
Schematic illustration of cell wall structures of microbial pathogens. A: Gram-negative
bacteria. B: Gram-positive bacteria. C: Fungal cells.

Figure 4.
Chemical structures of PSs described in this review. (A) Cationic phthalocyanine, Zn-PC-Me; (B) Hydroxygallium(III) 2,3,9,10,16,17,23,24-octakis-[3-(N-methyl) -pyridyloxy]-phthalocyanine octaiodide; (C) Toluidine blue O; (D) 3,7-Bis(N,N-dibutylamino) phenothiazinium bromide, PPA904; (E) 5-Phenyl-10,15,20-tris(N-methyl-4-pyridyl)-porphine trichloride, Sylsens B; (F) Rose Bengal; (G) Riboflavin; (H) Curcumin; (I) Polyethylenimine chlorin(e6), PEI-ce6; (J) Merocyanine 540; (K) Poly-S-lysine porphyrin conjugate, pL-TMPP; (L) Functionalized fullerene, C60(>ME1N6+C3); (M) Tri-meso (N-methyl-pyridyl), meso (N-tetradecyl-pyridyl) porphine, C14.
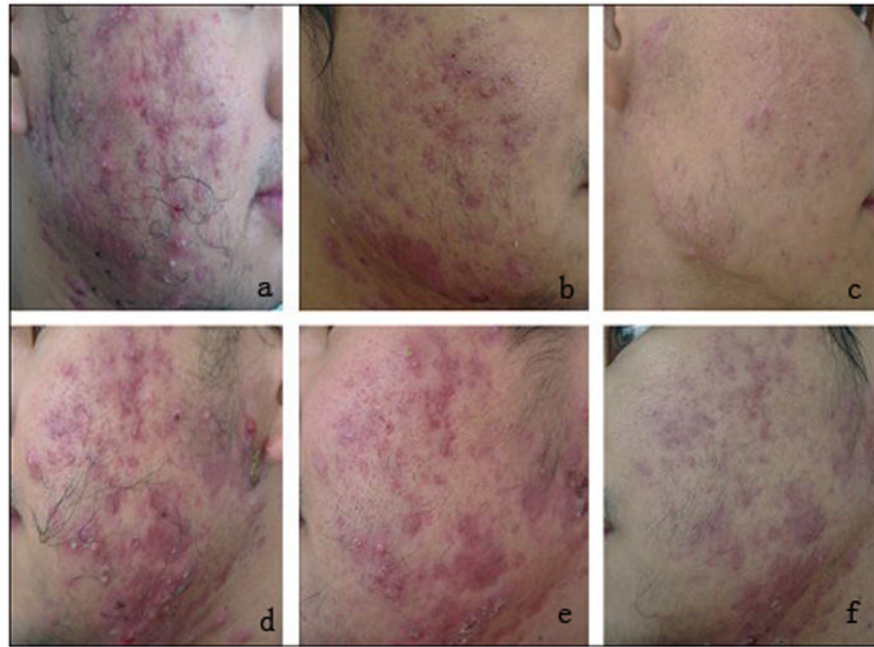
Figure 5.
Improvements of severe inflammatory acne treated by 15% ALA-PDT (a c) and control (only red light alone) (d f). (a, d) before treatment; (b, e) 2 weeks after four consecutive treatments; (c, f) at follow-up visit after 24 weeks [213].

Table 1

Studies of UVC irradiation for inactivation of bacteria/fungi *in vitro*

Light Source	Radiant Exposure	Bacterial/Fungi species/strains	Inactivation efficacy	Reference
254nm UVC	15.54 mW/cm^2	MRSA, VRE antibiotic-susceptible strains of *S. aureus* and *E. faecalis*	Illuminated 5 seconds, 99.9% MRSA and VRE inactivation; illuminated 9 seconds, 100% MRSA inactivation; illuminated 45 seconds, 100% VRE inactivation	5
254nm UVC	5mW/cm^2	MRSA, *Streptococcus pyogenes*	Illuminated 5 seconds, methicillin-resistant, coagulase-negative *Staphylococcus* and *Streptococcus pyogenes* inactivation; Illuminated 15 seconds, methicillin-susceptible *S. aureus* and *Enterococci* species inactivation	6
265nm UVC	1.93 mJ/cm^2	*S. aureus, E. coli, Pseudomonas aeruginosa, S. pyogenes*	Illuminated 1 seconds, 100% inhibition for all strains	7
254nm UVC	1500mJ/cm^2	catheter biofilms of *E. coli*, coagulase-negative *Staphylococcus, E. faecalis, Streptococcus, P. aeruginosa, Coryneforms*	Mean killing rates of the bacteria in catheter biofilms were 89.6% (11.8 mJ/cm^2), 98% (47 mJ/cm^2) and 99% (1500 mJ/cm^2)	8
254nm UVC	120mJ/cm^2	*Trichophyton rubrum, T. mentagrophytes, Epidermophyton floccosum, Microsporum canis.*	3–5 log10 of fungal inactivation	9
UVC	15.54 mW/cm^2	bacteria (*P. aeruginosa* and *Mycobacterium abscessus*) and fungi (*Candida albicans, Aspergillus fumigatus*)	Illuminated 3–5 seconds, 99% bacteria inactivation; Illuminated 15–30 seconds, 99% fungi inactivation	10

Table 2

Summary of representative clinical applications of antimicrobial PDT

Disease	Causative microorganism	Site of infection	Photosensitizer	No. of patients	Treatment outcome	Ref.
Localized bacterial infection	Gram (+)/Gram (−) bacteria	Leg ulcer, diabetic foot lesions	PPA904	10	Up to 2-log reduction in bacterial load was achieved after a single PDT.	270
	MRSA	Leg ulcer, diabetic foot lesions	PPA904	32	Bacterial counts were significantly lower after PDT. No side effects were experienced.	270,271
	Gram (+)/Gram (−) bacteria	Leg ulcer, diabetic foot lesions	PPA904	48	PDT was used once a week for 12 weeks. Interim data on 24 patients showed that 4 of 12 ulcers in the PDT group closed completely, compared with 0 of 12 ulcers in the placebo group.	270,271
Leishmaniasis	*L. major*	Skin	ALA	11 patients (32 lesions)	Completely healing was seen after one or two PDT sessions.	140
	L. major	Skin	ALA	20 patients (31 lesions)	3 months after PDT, 29 lesions were completely healed.	149
Acne	Acne vulgaris	Skin/sebaceous gland	Indole-3-acetic acid (IAA)	25	Numbers of both inflammatory and non-inflammatory acne lesions were significantly decreased. No adverse effects were observed except for transient pruritus in one patient.	272
	Acne vulgaris	Skin/sebaceous gland	ALA	78	22% of patients showed excellent improvement after one PDT session and another 34% showed excellent improvement after two PDT sessions. The rest (44%) required three PDT sessions to further reduce the number and size of residual lesions. Adverse effects were minimal.	273
Viral infection	Herpes simplex virus	Skin	MB	4	No significant acute side effects were noted and the lesions healed rapidly.	274
	Papillomavirus	Cervical condylomata	ALA	56	The overall complete remission rate of 1–4 sessions of PDT was 98.2% and the corresponding HPV clearance rate was 83.9%. Ten cases showed complete removal of cervical lesions and HPV infection after a single PDT. Adverse effects were minimal.	275
Gastric infection	*H. pylori*	Stomach	ALA	13	A maximum eradication effect was achieved at 4 h post-irradiation when 85% of biopsies in the monochromatic and 66% in the white light exposed zones, and 58 and 33% in the respective control zones were HP-negative.	276
Dental infection	*A. actinomycetemcomitans*	Dental pockets and gingival tissue	Phenothiazine chloride	10	PDT was effective in reducing numbers of *A. actinomycetemcomitans* than scaling and root planing.	277

Disease	Causative microorganism	Site of infection	Photosensitizer	No. of patients	Treatment outcome	Ref.
Onychomycosis	*T. rubrum*	Tonail	ALA	30	After one year, 13 patients (43.3%) were cured. The remaining patients had changes compatible with dermatophyte infection covering more than 10% of the nail plate.	207

Table 3

Studies on the *in vitro* antimicrobial effect of blue light

Light Source	Radiant exposure	Bacterial species/strains	Inactivation efficacy	Ref
405-nm diode laser	20 J/cm^2	*H. pylori*	>99.9%	225
405 nm light-emitting diode	15 J/cm^2 at lamp aperture	*P. gingivalis*	>75%	233
380–520 nm broadband light	4.2–42 J/cm^2	*P. gingivalis, P. intermedia, P. nigrescens, P. elaninogenica, S. constellatus*	*P. intermedia* and *P. nigrescens*: >5 log$_{10}$ at 4.2 J/cm^2; *P. melaninogenica*: >5 log$_{10}$ at 21 J/cm^2; *P. gingivalis*:1.83 log$_{10}$ at 42 J/cm^2	278
400–500 nm blue lamps	260 and 1300 mW/cm^2 for up to 3 min	*P. gingivalis, F. nucleatum, S. mutans, E. faecalis*	The minimal inhibitory dose for *P. gingivalis* and *F. nucleatum* was 16–62 J/cm2, for *S. mutans* and *S. faecalis* was 159–212 J/cm^2	279
405 and 470 nm light	15 J/cm^2	*S. aureus, P. aeruginosa*	*S. aureus*: 90% at 405 nm, 62% at 470 nm; *P. aeruginosa*: 95.1% at 405 nm, 96.5% at 470 nm	280
407–420 nm		five *P. acnes* strains	decreased by 15.7% immediately and 24.4% at 60 min after the irradiation.	231
407–420 nm	75J/cm^2	*P. acnes*	less than 2-log$_{10}$ units (99%) illuminated once; decreased by 4-log10 units (99.99%) after two illuminations and by 5-log$_{10}$ units (99.999%) after three illuminations	281

Table 4

Studies on blue light therapy for acne vulgaris

Light source	Clinical trial type	Acne type	Treatment regimen	No. of patients/acne location	Total follow-up time	outcome	Side-effects	Ref
407–420 nm metal halide lamp	open clinical trial	Mild to moderate acne	twice a week up to 5 weeks	30	during the trial period (0, 1, 3 and 5 weeks) and at 1 month after the final treatment	Acne lesions were reduced by 64%.	Two patients experienced dryness.	281
Handle 412nm LED	prospective, single-center, open-label study	Mild-to-moderate, including inflammatory or non-inflammatory	$2J/cm^2$/day full-face treatment or $29J/cm^2$/day localized spot treatment, twice daily at home	32/facial acne	eight weeks	100 percent of subjects considered their overall appearance was improved, clarity (97%), radiance (73%), tone (80%), texture (80%), and smoothness (83%)	Three adverse events: minimal transient skin dryness and minimal transient hyperpigmentation	282
home use Blue-light LED	IRB approved randomized self-control study	inflammatory	Four Treatments were conducted twice daily in the clinic	30/facial acne	followed-up till resolution	significant reduction in lesion size and lesion erythema as well as the improvement in the overall skin condition controlled with the placebo	NA	283
hand-held, Blue-light LED	open clinical trial	mild-to-moderate inflammatory acne	a daily dose of ~29 J/ cm^2, twice daily	33/facial acne	eight weeks	90% improvements	NA	284
407 to 420 nm LED	A prospective, randomized, open and comparative study	inflammatory acne lesions grades II or III	underwent 8 sessions of light therapy for 15 minutes each, twice a week, within minimum intervals of 48h	60/facial acne	4 weeks	The improvement achieved by the blue light was the same as the one with benzoyl peroxide	23.3% patients with mild desquamation and/or dryness	285
415nm LED	open-label study		48 J/cm^2, receiving two treatments of 20 minutes per week for a period of 4–8 weeks	45	2, 4 and 8 weeks after treatment	mean improvement score was 3.14 at 4 weeks and 2.90 at 8 weeks		286
blue LED, peak wavelength	open-label study	mild to moderat e acne	Over 4 weeks, patients	30	at weeks 5, 8 and 12	An overall effect on inflammatory		287
409–419 nm			received eight 10- or 20-minute light treatments			counts was observed at week 5, and a statistically significant decrease in inflamed counts		

Light source	Clinical trial type	Acne type	Treatment regimen	No. of patients/acne location	Total follow-up time	outcome	Side-effects	Ref
						was detected at the week 8 assessments, which continued to week 12.		