

# Antibacterial photodynamic therapy in dermatology

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Photodynamic therapy (PDT) appears to be endowed with several favourable features for the treatment of localized microbial infections, especially after the advent of cationic photosensitising agents (phenothiazines, *meso*-substituted porphyrins, polylysine-bound chlorins) which properly interact with the outer wall at the surface of several types of bacterial and yeast cells, increase their permeability, and allow significant amounts of photosensitizer to be accumulated at the level of the cytoplasmic membrane. These photosensitisers are characterized by a broad spectrum of activity, being effective toward both wild strain

and antibiotic-resistant gram-positive and gram-negative bacteria and yeasts. In general, extensive eradication of pathogens can be achieved under mild irradiation conditions, such as short incubation times and low fluence-rates, which guarantees a high degree of selectivity in comparison with the main constituents of host tissues, such as keratinocytes and fibroblasts. Moreover, the photosensitised inactivation of microorganisms is typically a multi-target process; as a consequence, the selection of photoresistant microbial strains is very unlikely and has not been experimentally observed so far. Possible initial targets

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of antimicrobial PDT applications include periodontal diseases, impetigo, atopic dermatitis, acne vulgaris, infected wounds, and superinfected psoriatic plaques.

## Introduction

Photodynamic activity of chemical compounds towards microorganisms was first published more than 100 years ago. Oskar Raab observed that toxicity of acridine hydrochloride against *paramecia caudatum* was dependent on the amount of light which was incident on the experimental mixture.<sup>1</sup> In addition, his teacher H. von Tappeiner, who worked with the same species, reported that the toxic effects in the presence of light are not due to heat.<sup>2</sup> In his assumption chemical activity of light is always linked to absorption. One part of absorbed light was converted from one wavelength to another. After further experiments in order to exclude direct influence of light, von Tappeiner coined the term 'photodynamic reaction' in 1904.<sup>3</sup> Additional experiments could show the involvement of oxygen in killing the bacteria, because antibacterial activity of fluorescent dyes against the facultative anaerobic species *Proteus vulgaris* could not be demonstrated in the absence of oxygen. At that time this new approach was applied to the skin of dermatologic patients. A. Jesionek and H. von Tappeiner observed good results in the treatment of psoriasis, lupus vulgaris, and skin cancer using a topical application of 5% eosin solution.<sup>4</sup> Another important step in the history of photodynamic therapy was in October 1912, when Friedrich Meyer-Betz, a German physician, performed a heroic self experiment. He injected himself 200 mg of hematoporphyrin and irradiated his forearm with a Finsen light source. Afterwards he observed an ulceration at the site of irradiation. However, after accidental exposure to sunlight outside the clinic a massive phototoxic reaction occurred with swelling and burning sensations even outside the irradiation site.<sup>5</sup>

Since the middle of the last century, antibacterial photodynamic therapy was forgotten because of the discovery of antibiotics. The first of them was penicillin, discovered by Alexander Fleming in 1928. The discovery of penicillin was the beginning of the *Golden Age* of antibiotics. More than 10 years of work was required by H. Florey and E. Chain before mass-produced penicillin made its clinical debut in the 1940s; virtually all strains of *S. aureus* were susceptible. The rapid isolation of further antibiotics, like streptomycin, chloramphenicol and tetracycline soon followed, and by the 1950s, these and several other antibiotics were clinically used. However, resistance to penicillin by penicillinase was recognized almost immediately after the first test in patients in 1944. Already in the late 1950s, 50 percent of all *S. aureus* strains were resistant against penicillin. Thus, the antibiotic methicillin was released in 1960 followed rapidly by the development of resistant strains of *S. aureus* in 1961. The problem is further aggravated by the lack of development of new antibiotics since the 1960s; only recently a new class of antibiotics was released, namely the oxazolidinones. The coagulase-positive *S. aureus* as well as both coagulase-negative *S. epidermis* and *S. hemolyticus* exhibit the capacity of developing resistance to each new generation of licensed antibiotics. Due to resistance to all beta-lactam antibiotics, vancomycin, a glycopeptid antibiotic, remained as last line of defence against gram (+) bacteria. However, in 1996, the first clinical isolate of a methicillin-resistant *S. aureus* with reduced susceptibility against vancomycin (MIC = 8 µg ml<sup>-1</sup>; vancomycin intermediate resistance type) was reported from Japan.<sup>6</sup> A few years later even, clinical infections caused by vancomycin-intermediate *S. aureus* (VISA) were confirmed in the United States.<sup>7,8</sup> The first documented case of infection caused by vancomycin-resistant (VRSA) *S. aureus* (MIC ≥ 32 µg ml<sup>-1</sup>) was reported in July 2002.<sup>9</sup>

Totally the worldwide rise in antibiotic resistance has driven research to the development of new antibacterial strategies. In particular, the emergence of mupirocin (Bactroban®) resistance

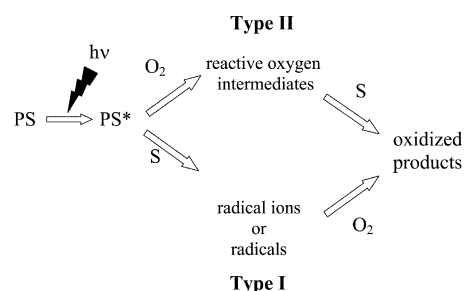
of MRSA *Staphylococcus aureus* make alternatives to standard antibiotic treatment of skin infections necessary.<sup>10</sup>

But it was not until the 1990s that topical application of photodynamic reactive dyes was resumed. A milestone in the history of topical photodynamic therapy in dermatology was the years 1999. 5-aminolevulinic acid (ALA), a precursor of the heme biosynthesis inducing protoporphyrin IX, was the first topical photodynamic therapy agent to receive regulatory approval by the U.S. FDA in 1999 for the treatment of actinic keratoses in dermatology. In recent decades, different classes of chemical compounds with photoactive properties were tested with different results against gram (+) as gram (–) bacteria.<sup>11–13</sup> In general, antibacterial photodynamic therapy utilizes visible or ultraviolet light in combination with a photosensitising agent to induce a phototoxic reaction which results in cell damage or death.

The purpose of this review is to summarize the knowledge of antibacterial photodynamic therapy regarding the application in dermatology.

## I General photobiological and photochemical aspects

Processes in which absorption of light by a chromophore (photosensitizer) induce chemical changes in another molecule (substrate) are defined as photosensitizing reactions (Fig. 1). The details have been described already.



**Fig. 1** Pathway of type I and Type II reaction of light absorbing photosensitizer. After light activating of the ground state of photosensitizer (PS), activated form of PS\* can follow two alternative pathways via reactive singlet oxygen (<sup>1</sup>O<sub>2</sub>), hydrogen peroxide, hydroxyl radical (type II) or organic substrate (S) (type I). The intermediates react rapidly with their surroundings: cell wall, cell membrane, peptides, nucleic acids.

In summary, the photodynamic activity to induce cell damage or death is determined by five important photophysical/photochemical properties including (I) an overall lipophilicity and ionisation of the photoreactive dyes, (II) the molecular extinction coefficient  $\epsilon$ , (III) quantum yield of the triplet state formation  $\Phi_T$ , (IV) redox potentials of the excited states of the PS<sup>s</sup><sub>red</sub> or PS<sup>t</sup><sub>red</sub>, if the reaction follows the type I mechanism or (V) the quantum yield of the singlet oxygen <sup>1</sup>O<sub>2</sub> generation, if the reaction occurs by a type II photosensitization.<sup>14</sup>

A large number of different compounds with photodynamic activity are now available (Table 1). First of all the synthetic non-porphyrin compounds have demonstrated photosensitising ability, like the phenothiazine dyes: methylene blue and toluidine blue. Next macrocyclic molecules have shown phototoxicity, like phthalocyanines and the metal containing porphyrines as well as the metal free porphyrines. Another group of dyes belongs to the naturally occurring photosensitizers. Psoralens (furanocoumarins) and perylenequinonoids are two examples of natural products which originally act in plants as chemical defence substances against microbial or eukaryotic organisms. In fungi furanocoumarins normally facilitate the parasitization of plants.<sup>12</sup>

**Table 1** Overview of photosensitizers

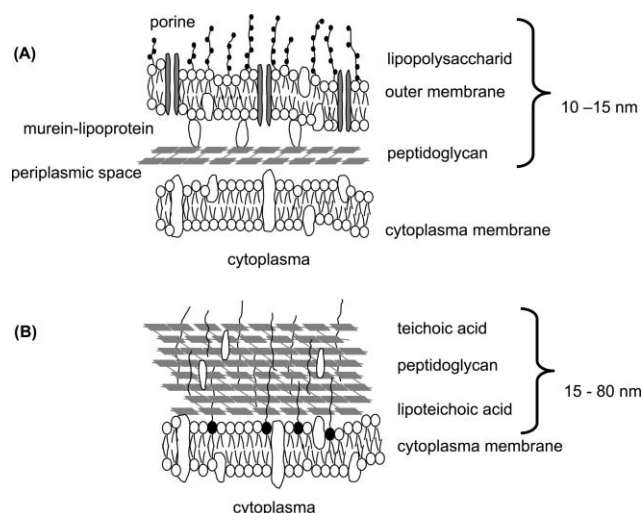
Compound group	Name	Site of action by prokaryotic cells	References
Phenothiazines	Methylene blue	DNA interaction	42
	Toluidine blue	Membrane	142
Macrocyclic molecules	Acridine	DNA interaction	12
	Phthalocyanine	Membrane/cytosolic	29
	Porphyrin		
Natural products	Furanocoumarin	DNA intercalation	143
	Perylenequinonoid/hypericin	Inhibitor of protein kinase C	144

## II Photoinactivation of microorganisms: general aspects

Photoinactivation of gram (+) as gram (–) bacteria is based on the concept that certain photosensitizers (PS) can accumulate in significant amounts in or at the cytoplasmic membrane, the critical target to induce a irreversible damage in bacteria. The positive charge of the PS appears to promote a tight electrostatic interaction with negatively charged sites at the outer surface of the bacterial cell.<sup>15</sup>

In recent years it has been shown that there is a difference in susceptibility to antibacterial PDT between gram (+) and gram (–) bacteria.<sup>15–17</sup> Anionic and neutral photosensitizers were found to bind efficiently to gram (+) bacteria and to induce growth inhibition or killing by PDT. On the other hand these PS bind only to the outer membrane of gram (–) microorganisms and these bacteria were not killed. Further studies have established that gram (+) bacteria are very sensitive to the photosensitising action of anionic or neutral PS absorbing visible light. However, gram (–) bacteria show a remarkable resistance to antimicrobial PDT.<sup>11,18,19</sup> Growth inhibition of *E. coli* by porphyrin-photosensitization was possible only in the presence of the nona-peptide polymyxin or Tris-EDTA, which are membrane disorganising substances.<sup>17</sup> This is due to the different outer membrane structure of gram (+) and gram (–) bacteria (Fig. 2). Gram (+) bacteria are characterized by the presence of a 40–80 nm thick outer peptidoglycan wall with no significant amount of lipids or proteins. This murein sacculus contains up to 100 peptidoglycan layers, which is much thicker than that of gram (–) bacteria. However, this network does not represent a permeability barrier because it is more or less porous. Measurements of the penetration of polysaccharides, antimicrobial peptides and glycopeptides have shown that molecules of molecular weight in the range of 30 to 57 kDa can diffuse through this murein sacculus.<sup>20,21</sup> In general, resistance against the penetration of antibiotics is related to mechanisms concerning active efflux, changes in the target site, or inactivation.<sup>22</sup>

In contrast, gram (–) bacteria contain an additional membrane layer in the cell wall architecture, which is located outside the peptidoglycan layer and shows an asymmetric lipid structure composed by strongly negatively charged lipopolysaccharides (LPS), lipo-proteins and proteins with porin function (Fig. 2). Hydrophilic compounds (<600 to 700 Da for *E. coli*) can diffuse through the outer membrane using the porins, which are characterized as aqueous channel-forming proteins.<sup>23,24</sup> Hence, the outer membrane acts as a very effective permeability barrier, making the gram (–) bacteria resistant against host cellular and humoral defence factors. Furthermore, the outer membrane triggers mechanisms of resistance against many antibiotics, which are normally sensitive to gram (+) bacteria.<sup>25,26</sup> There are several mechanisms which together achieve an efficient resistance against antibiotics (like low permeability membranes, altered porins and active efflux). One such mechanism is the active efflux of drugs out of the cell in order to minimize the concentration of the antibiotic inside the cell. Antibiotic resistant *E. coli* have reinforced the production of TolC, an outer membrane protein, which is part of a drug-efflux system.



**Fig. 2** Schematic representations of the arrangement of the cell walls of gram-negative (A) and gram-positive (B) bacteria. Gram (–) bacteria cell wall consists of a thin, inner wall composed of 2–3 layers of peptidoglycan (2–3 nm thick), a periplasmic space and an outer lipid bilayer (7 nm). The outer membrane contains phospholipids, lipoproteins, lipopolysaccharides (LPS) and proteins like porins (A). Gram (+) bacteria cell wall appears as a 15–80 nm thick cell wall composed of up to 100 interconnecting layers of peptidoglycan (B). Teichoic acids are interwoven in the peptidoglycan layers. Some have a lipid attached (lipoteichoic acid). Also proteins are ingrained in the peptidoglycan layers.

This transport system is known to confer resistance to *E. coli* against antibiotics like *e.g.* chloramphenicol, ciprofloxacin and novobiocin.<sup>27</sup>

Porphyrins with a molecular weight higher than 1 kDa cannot diffuse through the narrow porin channels which selectively allow the influx of low molecular weight nutrients. As a consequence the generation of the induced reactive oxygen species, in particular singlet oxygen occurs only at the outer cell membrane. This is supported by the short diffusion length of singlet oxygen ( $^1\text{O}_2$ ), because  $^1\text{O}_2$  generated by haematoporphyrin cannot induce DNA strand cleavages inside of *E. coli*.<sup>28</sup> A pioneering study on the molecular pathways involved in photosensitized inactivation of bacteria has been recently published.<sup>29</sup>

Photosensitivity of gram (–) bacteria can be enhanced by the addition of biological or chemical molecules, *e.g.* the nona-peptide polymyxin or Tris-EDTA, which are known to alter the native consistence of the outer membrane, thereby enhancing its permeability and facilitating the penetration of phototoxic molecules to the cytoplasmic membrane.<sup>30</sup> The addition of Tris-EDTA to gram (–) bacteria removes the divalent cations (*e.g.*  $\text{Ca}^{2+}$ ,  $\text{Mg}^{2+}$  ions) which are present in large numbers to stabilise adjacent negative charged LPS molecules at the outer membrane. Thus the neutralisation of negative charges is prevented. Hence the onset of electrostatic repulsion promotes the release of up to 50% of the lipopolysaccharides into the medium, thereby allowing the penetration of molecules with molecular weights as high as 1.000–2.000 dalton to the inner cytoplasmic membrane or inner cellular compartments.<sup>23</sup>



Bertoloni *et al.* could show that addition of Tris-EDTA prior to photosensitization with hematoporphyrin is effective in inactivation of *E. coli* and *Klebsiella pneumoniae*.<sup>36</sup> The exposure of gram (–) bacteria to the action of low concentrations of non-toxic polycations (e.g. nonapeptid polymyxin B or EDTA) displace divalent cationic counterions, because polymyxin B tends to undergo an electrostatic binding with the negatively charged cell surface molecules. By that way the physical arrangement of the ordered lipid layer is heavily altered with less densely packed hydrocarbon lipid chains.<sup>31,32</sup> As a result, the barrier properties of the outer membrane are strongly reduced and a variety of antibiotics and detergents can diffuse towards the plasma membrane.<sup>33</sup> The addition of polymyxin B reduces the minimal inhibitory concentration (MIC) values of novobiocin and erythromycin toward *E. coli* and *K. pneumoniae*.<sup>34</sup> Therefore, the nonapeptide polymyxin B seems to be particularly active as a disorganizing agent for the outer membrane of gram (–) bacteria cells.

Different chemical classes of positively charged PS, including phenothiazines (methylene blue and toluidine blue), phthalocyanines and porphyrins, have been successfully tested as photoinactivating agents against gram (+) and gram (–) bacteria so far.<sup>12,15,16</sup> These reports have shown that in general photosensitizers with an overall cationic charge as well as *meso*-substituted cationic porphyrins and water soluble cationic zinc phthalocyanines can efficiently kill gram (–) bacteria by photosensitization even in the absence of additives.<sup>35</sup> An explanation for the photoinactivation of gram (–) bacteria by porphyrins may be the impairment of cellular functions due to the localisation of the molecules because of their positive charges, since *meso*-substituted, but negatively charged porphyrins have not shown toxicity against gram (–) bacteria,<sup>36</sup> despite the generation of singlet oxygen by both porphyrins.<sup>37</sup> Certainly, the *meso*-substitution itself is unlikely to be the relevant factor, because cationic phthalocyanines, which are structurally unrelated to *meso*-porphyrins, show similar photobiological properties against gram (+) and gram (–) bacteria.<sup>16</sup> Thus other parameters must be relevant to explain the 'relative' resistance of gram (–) bacteria against photosensitization. On the other hand it is possible to kill *E. coli* in the same way as gram (+) bacteria by using 10-fold higher concentrations as well as three times longer illumination times.<sup>15,16,36</sup>

A possible explanation for the cationic PS uptake by *E. coli* is the fact that in nature some cationic compounds are taken up through the so called self-promoted uptake pathway.<sup>38,39</sup> These molecules have a 2–4 orders of magnitude higher affinity to binding sites on surface LPS molecules than the divalent cations  $\text{Ca}^{2+}$  as  $\text{Mg}^{2+}$ , and they competitively displace these cations. The displacing of  $\text{Ca}^{2+}$  as  $\text{Mg}^{2+}$  leads to a reorganization of the outer membrane structure and permeabilization of the outer membrane to various antibiotics and hydrophobic molecules.

In an early study Ehrenberg *et al.* found that *E. coli* spheroplasts, which were lacking the outer membrane and cell wall, were able to bind metal free porphyrins to the same extent as gram (+) bacteria.<sup>40</sup> Nevertheless, *E. coli* spheroplasts were not killed by these porphyrins and light. According to these findings it is not sufficient to disturb the outer-membrane structure alone, but the cytoplasmic membrane must be disrupted as well to kill gram (–) bacteria to the same extent as gram (+) pathogens. This observation indicates that inactivation of gram (–) bacteria depends on the chemical structure of each molecule as well as its ability to penetrate the outer membrane and to reach the cytoplasmic membrane.

In general, photosensitization of *S. aureus*, *E. coli* and *P. aeruginosa* has shown alterations of the ultra-structure of the cells, e.g. disordered cell wall structure, elongated cells connected together without separation of the daughter cells and different low density areas in the cytoplasm.<sup>17,41</sup> However, there is some evidence that treatment of bacteria with PS and light leads to DNA damage.<sup>42</sup> But it may not be the prime cause of bacterial

cell death, because *D. radiodurans*, which is known to have a very efficient DNA repair mechanism, is easily killed by photosensitization.<sup>43</sup>

During the last ten years some major advances in the field of antimicrobial PDT have been made, which are characterized by the following points:

An overall short time of antibacterial PDT is due to a very fast uptake of the photosensitizer agents by bacteria (few minutes) followed by a relatively low intensity (e.g. 40–100 mW cm<sup>–2</sup>) yielding a significant reduction of pathogens (2–4 log reduction of growth curves).

Availability of a broad spectrum of photosensitizers, e.g. phenothiazines, phthalocyanines or porphyrins, which have shown a significant antibacterial activity against gram (+) as well as gram (–) bacteria.

Applicability against antibiotic resistant bacteria independent of their antibiotic resistance pattern. This property is important regarding the repeated treatment of chronic and/or recurrent infections.

Lack of induction of resistance after multiple treatments. Ongoing studies have shown that at least up to fifteen generations of porphycene-photosensitized *S. aureus* and *E. coli* developed no resistance to PDT (Jori, unpublished results).

Lack of mutagenicity. One potential advantage of PDT over UVA-treatment causes that PDT may not be intrinsically carcinogenic.

Demonstration that PDT can also destroy virulence factors associated with bacterial infections.<sup>44</sup>

The challenge in antimicrobial PDT is to find a therapeutic window *in vivo* where bacteria can be killed without cytotoxic effects to the surrounding tissue. The MIC values of an appropriate photoreactive dye must induce a three log step growth reduction (cfu ml<sup>–1</sup>) *in vivo* to kill bacteria (≥99.9%) and must be lower than those determined for cutaneous cell types.

If all points are fulfilled, antimicrobial PDT will offer a safe alternative to conventional antimicrobial treatment *in vivo*.

### III Colonization of normal human skin by micro-organisms

The normal human skin is colonized by a large number of different microorganisms (bacteria and yeasts) which can be determined in modest numbers for long periods. The resident aerobic bacteria on the skin surface consists of gram positive cocci of *Staphylococcus* species, *Micrococcus* species and the gram positive rods, the *coryneforms*. The obligate anaerobes and/or microaerophilic bacteria are mainly gram positive *Propionibacterium* species. In contrast to *coryneforms*, they are able to tolerate microaerophilic conditions. These anaerobes inhabit the skin in the deeper parts of the hair follicles. The only significant gram negative residents are *Acinetobacter* species.

Within the genus *Staphylococcus*, 10 different species have been isolated from healthy skin.<sup>44</sup> The coagulase-negative staphylococci (e.g. *S. epidermidis*, *S. hominis*) are the most important organisms of the normal skin flora and contribute to resistance against colonization by pathogenic bacteria. That is, because *S. epidermidis* binds to the same keratinocyte receptors as virulent *S. aureus* and inhibits the adherence of virulent *S. aureus*.<sup>45,46</sup> Primary infection of healthy people by *S. epidermidis* causes minor skin diseases, like folliculitis. However, *S. epidermidis* may cause infections of wounded skin, in particular around surgical implants.

The coagulase-positive species *Staphylococcus aureus* should not be considered as a resident on normal skin, however it is frequently found in the anterior nares.

The *Corynebacterium* species are difficult to distinguish from each other by conventional taxonomic methods and chemotaxonomic methods. *Corynebacteria* are gram- and catalase-positive pleomorphic rods. Up to now

the aerobic coryneforms are divided into four species: *C. bovis*, *C. minutissimum*, *C. xerosis* and *C. hofmani*.

*Propionibacteria acnes* and *P. granulosum* are now generally confirmed as members of the resident flora in adults. These two species are widespread on the skin, but particularly associated with follicles. Another *Propionibacteria*, *P. avidum*, is found in humid sites of the skin, in particular in the axillae and groins. Its pathogenic potential on the skin is unclear.

Another opportunistic resident of normal skin belongs to the species of yeasts. Yeasts of the genus *Malassezia* (previously called *Pityrosporum*) are round or oval monopolar-budding yeasts in the stratum corneum of humans.<sup>47</sup> *Malassezia furfur* organisms are present in 75 to 98% of healthy individuals<sup>48</sup>

#### IV Infection of the skin by microorganisms

Certain skin and soft tissue infections, to be intended as the deposition and multiplication of bacteria in the tissue, may lead to severe complications such as sepsis. Staphylococcal skin infections are commonly seen in both healthy and immunocompromised persons. The coagulase-positive species *S. aureus* is well defined as a human opportunistic pathogen. Certain isolates of *S. aureus* are able to produce more than 30 different extracellular proteins, which are involved in the pathogenesis of staphylococcal induced diseases.<sup>49</sup> For example *Staphylococcus aureus* is the causative agent in up to 75% of primary pyodermas.<sup>50</sup> Additional risk factors for secondary infection with *S. aureus* are pre-existing abnormalities in skin structure such as tissue injuries, exudative dermatitis, as well as diabetes mellitus and neoplasms.<sup>50</sup> Also during chronic inflammatory skin diseases such as atopic dermatitis and psoriasis, the involved dermis is known to be colonized with *S. aureus*.<sup>51,52</sup> The main skin diseases associated with *S. aureus* are shown in Table 2. However, these diseases are not only caused by *S. aureus* but also by a mixture of other aerobic and anaerobic organisms: Gram-positive *Streptococcus pyogenes* and *Enterococci spp.* (see Table 3), gram-negative bacteria such as *Pseudomonas aeruginosa* and *Enterobacteriaceae* and the anaerobes species *Bacteroides fragilis* are frequently isolated.<sup>53–57</sup>

**Table 2** Cutaneous diseases and *Staphylococcus aureus*<sup>a</sup>

Direct infection of skin and adjacent tissues	Secondary infection	Effect of bacterial toxin SEA, SEB, SEC, TSST-1
Carbuncle	Eczema	Staphylococcal scalded skin syndrome
Ecthyma	Ulcers	Toxic shock syndrome
Folliculitis		Staphylococcal scarlatina
Furunculosis		Psoriasis
Impetigo		Atopic dermatitis
Occasionally in cellulites		
Sycosis		

<sup>a</sup> Modified from ref. 145.

**Table 3** Cutaneous diseases and *Streptococcus pyogenes*<sup>a</sup>

Direct infection of skin and adjacent tissues	Secondary infection	Effect of bacterial toxin: SPEA, SPEB, SPEC
Blistering distal dactylitis	Eczema	Scarlet fever
Cellulitis	Infestations	Toxic shock like syndrome
Ecthyma	Ulcers	Acute guttate Psoriasis
Erysipelas		
Impetigo		
Necrotizing fascitis		
Streptococcal ulcers		

<sup>a</sup> Modified from ref. 145.

**Table 4** Resident microbial flora of healthy and atopic skin<sup>a</sup>

Microbial skin flora	Normal skin	Atopic dermatitis
<i>Micrococcaceae</i>		
<i>Staphylococcus aureus</i>	—	+++
Coagulase-negative <i>Staphylococci</i>	+	++
<i>Coryneform</i> organisms		
<i>Corynebacteria</i>	+	+
<i>Propionibacteria</i>	+	+

<sup>a</sup> From ref. 51.

Another known important pathogen apart from *S. aureus* relevant in dermatology belongs to the group A beta-hemolytic streptococci, *Streptococcus pyogenes*. It has the ability to cause a variety of skin diseases ranging from self-limited superficial skin infections to fulminant life-threatening soft-tissue destruction and necrotizing fasciitis.<sup>58,59</sup> Table 3 shows relevant skin diseases caused by *Streptococcus pyogenes*. Adherence of *S. pyogenes* is mediated by the CD46 receptor on keratinocytes.<sup>60,61</sup>

*Malassezia* infection occurs by a change from the saprophytic phase of *Malassezia furfur* to its pathogenic mycelian phase.<sup>62</sup> Several endogenous factors could be the cause of the transformation to the mycelian phase, such as sweating, greasy skin or immunosuppression as well as exogenous factors like high temperature and humidity. Five inflammatory diseases associated with *Malassezia furfur* are divided at least into two groups. The first group includes Pityriasis versicolor and *Malassezia* folliculitis. The second group includes atopic dermatitis, seborrheic dermatitis and psoriasis.<sup>63</sup> *Malassezia* folliculitis is a chronic disease characterized by pruritic follicular papules and pustules often associated with itching.<sup>64,65</sup> Pityriasis versicolor is a chronic disease of the skin characterized by various pigmentary changes.<sup>66,67</sup> It is often seen in AIDS patients.<sup>68,69</sup> Up to now the cause of seborrheic dermatitis is still unknown. Some authors discuss the role of an inadequate immune response against *Malassezia furfur* as the cause of seborrheic disease.<sup>70,71</sup> This relationship can be found in AIDS patients who have a reduced T-cell function, in a higher incidence of seborrheic dermatitis as well as a higher presence of *Malassezia furfur*.<sup>72,73</sup>

In the last two decades the treatment of cutaneous diseases, like atopic dermatitis, psoriasis and impetigo (Table 4), associated with bacterial pathogens were performed with frequently used antimicrobial agents including cephalosporins<sup>74</sup>  $\beta$ -lactam- $\beta$ -lactamase inhibitor combinations<sup>55</sup> or fluoroquinolones.<sup>56,75</sup> Since 1980 methicillin-resistant *S. aureus* (MRSA) emerged as a major clinical and epidemiological problem in hospitals.<sup>76</sup> In 10 European countries, the proportion of *S. aureus* isolates resistant to methicillin ranged from <1% in Scandinavia to >30% in Spain, France and Italy.<sup>77</sup> Furthermore the existence of multiple antibiotic resistant strains of *S. epidermidis* and the possible transmission of such a resistance-pattern to *S. aureus* reinforced the problem of MRSA strains. The infection with these multidrug-resistant pathogens is leading to increased morbidity, prolonged hospitalisation and may even become life-threatening.<sup>78</sup> In addition, localized skin and soft tissue infections may not need to be treated with systemic medication if an efficient alternative is available. Thus, the development of new strategies in antibiotic treatment becomes more and more important.

#### V Involvement of pathogens in skin diseases

The following clinical skin diseases with the involved multiresistant bacteria species should demonstrate the importance of developing possible alternatives for topical and/or systemical antibiotic therapy.

## a Treatment of wound infections

Primary topical antibacterial and antiseptic agents are indicated in both prophylaxis and the treatment of infection. One advantage of topical application of antibacterial agents is their low systemic absorption, consequently the reduced exposure of the commensal gastrointestinal flora to these antibiotics as well as the low systemic toxicity.<sup>79</sup> The principles of antimicrobial treatment of infected skin wounds are discussed extensively by Bowler *et al.*<sup>80</sup>

Today topical therapy with antibiotics has become unpopular because of the development of resistance.<sup>81</sup> Colsky *et al.* made a comparison of antibiotic resistance profiles using data collected from 1992 to 1996 from patients with skin wounds and revealed a marked increase in oxacillin and ciprofloxacin resistance in *S. aureus* and *P. aeruginosa*. Respectively, in leg ulcers an increase from 24 to 50% oxacillin resistance in *S. aureus* and from 9 to 24% ciprofloxacin resistance in *P. aeruginosa*, and in superficial wounds an increase of 24 to 36% ciprofloxacin resistance in *P. aeruginosa*.<sup>53,82</sup> This study demonstrates the rapid increase of antibiotic resistant bacterial pathogens due to the systemic use of antibiotics in dermatology and highlights the importance to search for alternatives. Wound infections are often treated with non antibiotics, such as iodine, however they are not specific and can cause host tissue sensitisation.

Hamblin and colleagues could show the use of a photochemical approach to destroy bacteria infecting a wound in an animal model without damaging the surrounding host tissue.<sup>83,84</sup> Following topical application of a chlorin(e6) photosensitizer conjugated with poly-L-lysine *E. coli* was rapidly eradicated. Similarly, porphyrins proved to be highly active for the eradication of *S. aureus* and selected viral pathogens in burn wound infections.<sup>85,86</sup>

## b Impetigo

Impetigo appears in two different classic forms: bullous (impetigo bullosa) and non-bullous (impetigo contagiosa). Bullous impetigo simply means that the skin eruption is characterised by clear fluid blisters (bullae). All cases of bullous impetigo are caused by coagulase-positive *Staphylococcus aureus*. Initially, the disease is triggered by exo-toxins (SEA, SEB, TSST-1) produced at the site of infection by *S. aureus* and can occur on intact skin.<sup>57</sup> Usually, impetigo bullosa appears initially as a very thin-walled vesicle, on previously intact skin that rapidly ruptures. The dissemination of such lesions results in a widespread redness with large blisters of the skin within 24–48 h. These blisters rupture easily and the result is exfoliation, which is named localised staphylococcal scalded skin syndrome.

Original impetigo contagiosa is a streptococcal infection of the skin in children aged 2 to 5 years.<sup>57</sup> Now, it is most often caused by *Staphylococcus aureus* and/or a combination of group A beta-hemolytic streptococci (GABHS) and *S. aureus*. Impetigo contagiosa is characterized as a superficial, intraepidermal, vesiculopustular infection. The microorganisms enter and colonize the damaged skin of the face or extremities directly by binding to sites on fibronectin.<sup>59</sup> In contrast, intact skin is resistant to colonization, because of the unavailability of fibronectin receptors for teichoic acid moieties on *Streptococcus pyogenes* and *S. aureus*.<sup>59</sup> After infection, the development of new lesions nearby may be also seen without an apparent rupture of the skin barrier. Therefore, these lesions emerged by bacterial damage to the keratinocytes underneath an intact epidermis.

Application of mupirocin (Bactroban®) ointment is the treatment of choice, and has been demonstrated to be as effective as oral antibiotics.<sup>87</sup> Particularly the emergence of mupirocin (Bactroban®) resistance of MRSA *Staphylococcus aureus* has shown that other alternatives than standard antibiotic treatment of skin infections are urgently needed.<sup>10</sup> In 1998, Rossney and Keane investigated the evidence of strain variation in MRSA populations. High-level mupirocin resistance (MIC >

1024 mg l<sup>-1</sup>) exhibit 4% of the MRSA isolates (*n* = 104) and 32% showed low-level resistance (MIC 8–24 mg ml<sup>-1</sup>).<sup>88</sup>

Zeina and co-workers<sup>89,90</sup> have shown that *S. pyogenes* can be killed by methylene blue photosensitization at concentrations lower than those used for treating other skin diseases, such as psoriasis. PDT effects on this kind of pathogens appears to be endowed with a high degree of selectivity since the rate of methylene blue-mediated inactivation of keratinocytes is about 200-fold slower.

## c Atopic dermatitis

The resident microbial flora of normal and atopic skin shows striking differences in terms of the presence of *Staphylococcus aureus* (Table 2 and Table 4). On normal skin the colonization rate of *Staphylococcus aureus* is between 2–25%, in contrast to the high residual rate found in patients with atopic dermatitis, ranging from 76% on unaffected areas up to 100% on acute lesions.<sup>51,68</sup> The typical feature in patients with atopic eczema is an abnormally superinfected skin by *S. aureus*. Up to 10<sup>6</sup> cm<sup>-2</sup> *S. aureus* have been described on lesions of atopic dermatitis and such a high number of bacteria could be assumed to be sufficient to explain the exudation and impetiginization seen in atopic dermatitis patients.<sup>91,92</sup> Moreover, the adherence of *S. aureus* to keratinocytes is increased in atopic dermatitis.<sup>93</sup> The degree of adherence is related to the progress of keratinisation. Furthermore the production of exotoxins with super-antigenic properties by *S. aureus*, which may cause T-cell activation, cytokine release and mast cell degranulation, will aggravate this inflammatory skin disease.<sup>94</sup> The staphylococcal enterotoxins A-D (SEA-D) as well as the toxic syndrome toxin-1 (TSST-1) have been found to be secreted by *S. aureus* isolated from the skin of up to 65% of atopic dermatitis patients who were colonized with this microorganism.<sup>95</sup> Above all the superantigen staphylococcal enterotoxin B (SEB) applied on intact skin from both normal subjects and patients with atopic dermatitis induces an inflammatory reaction.<sup>96</sup> SEB-treated skin areas demonstrated selective accumulation of specific T cells expressing a SEB-reactive T-cell receptor Vβ repertoire.<sup>97</sup>

There are different therapeutic approaches for localized atopic lesions.<sup>98</sup> Regarding the treatment with antibiotics, fusidic acid seems to be the drug of choice due to its inhibition of staphylococci, regardless of the resistance-pattern against methicillin or oxacillin.<sup>99</sup> Between 1989 and 1995 Speller *et al.* could show an increased resistance to methicillin, erythromycin, clindamycin, ciprofloxacin, gentamicin, trimethoprim and rifampicin, but a stable resistance to fusidic acid.<sup>100</sup> In the future, this may become a problem if only one effective antibiotic is available. However, another possibility to treat acute and chronic severe stages of atopic dermatitis with an impact on the bacterial colonization is conventional UVA/UVB therapy.<sup>101</sup> Yoshimura *et al.* demonstrated an anti-staphylococcal effect after UV therapy.<sup>102</sup> However, all non-responders to UVA1 therapy were characterized by a higher *Staphylococcus aureus* colonization rate as compared to responders.<sup>103</sup>

## d Psoriasis

Psoriasis is a multifactorial disease of still unknown aetiology. There are two clinical types of non-pustular psoriasis known: acute guttate psoriasis and chronic type I plaque psoriasis. Bacterial infections such as streptococcal infection is a well known exacerbating factor in acute guttate psoriasis.<sup>81,104–107</sup> In addition, in patients with chronic plaque psoriasis 50% harbour *S. aureus* on their skin.<sup>108</sup> Initially in guttate psoriasis the lesions are initiated by superantigen-producing streptococci. All *Streptococci* strains secrete streptococcal pyrogenic exotoxins (SPE), e.g. SPEC, which are known to stimulate the marked expansion of Vβ+ T-cells.<sup>109</sup> These findings together with previous results regarding superantigen T-cell activation can explain the T-lymphocyte activation by streptococcal superantigens.<sup>110</sup>



This in turn induces expansion of V $\beta$ 2+ T-cells and cutaneous localization that initiate the characteristic inflammatory reactions in psoriasis. Up to 10% of all circulating T-cells can be activated by superantigens.<sup>111</sup> However, there may be an association between streptococcal pharyngitis and psoriasis.<sup>112</sup> Induction of T-cell activation by streptococcal superantigens through streptococcal pharyngitis, induces a cutaneous localization and proliferation of V $\beta$ 2+ T-cells, which in turn initiates inflammatory processes.<sup>112</sup>

In addition, not only streptococcal but also staphylococcal superantigens are proposed as a possible antigen in chronic plaque type I psoriasis.<sup>113</sup> Data from Yamamoto *et al.* suggest that reactivity of PBMCs to staphylococcal enterotoxin B (SEB) may lead to the exacerbation and persistence of chronic plaque psoriasis by the induction of several inflammatory cytokines.<sup>114</sup> For example, TNF- $\alpha$  induces the production of intercellular adhesion molecule-1 (ICAM-1) by keratinocytes as well as an increased expression of IL-8 by keratinocytes, dermal fibroblasts and endothelial cells.<sup>115–117</sup> However, TNF- $\alpha$  increases the secretion of pro-inflammatory cytokines such as IL-1 and IL-6 by keratinocytes.<sup>118</sup> Thus, TNF- $\alpha$  is thought to play a key role in the pathogenesis of psoriasis concerning their immunomodulatory properties.

Photochemotherapy (psoralen plus ultraviolet (UVA treatment (PUVA)) is a very effective and widely used treatment modality of psoriasis.<sup>119,120</sup> A disadvantage of such multiple PUVA treatments is the possibility to increase the risk of developing skin cancer in patients with psoriasis: basal cell carcinoma, squamous cell carcinoma or even melanoma.<sup>121–123</sup>

Recently photodynamic therapy with topical application of 5-ALA followed by broad-band visible irradiation was tested in patients with chronic stage plaque psoriasis.<sup>124,125</sup> Selectivity of protoporphyrin IX accumulation in plaque psoriasis after topical application of 5-ALA and photobleaching during PDT has been established.<sup>125</sup> However, the clinical response of patients with plaque psoriasis after PDT with topical application of 5-ALA revealed no clear correlation between clearance of plaque areas and the delivered irradiation dose.<sup>126,127</sup> On the other hand a study using an ointment containing 10% of 5-ALA, which was applied topically to plaque lesions 5 h before irradiation, documented a beneficial effect of PDT in psoriasis.<sup>128</sup>

More recently, an open non-randomised phase I and II study in 20 patients with chronic plaque-stage psoriasis revealed that after intravenous administration of the photosensitizer verteporfin and subsequent irradiation all patients exhibited improved clinical response.<sup>129</sup>

These preliminary results are encouraging to develop new regimes of systemic application of photosensitizers without an associated prolonged photosensitivity. In future, the use of PDT with photosensitizer and polychromatic light to treat psoriasis might represent an alternative therapy to PUVA.

## e Acne vulgaris

Acne is a disease of the pilosebaceous follicles. The principal pathogenic factors in acne are: (1) abnormal follicular keratinization leading to plugging of the follicle; (2) increased sebum production under the follicular plug; (3) inflammation; (4) proliferation of *Propionibacterium ssp.* in the sebum. *P. acnes* and *P. granulosum* are found mainly in sebaceous areas of the skin. At present, the role of these ubiquitous bacteria in the pathogenesis of acne remains unclear, because there is a very weak association between the severity of acne and the number of *P. acnes* within superficial pilosebaceous follicles.<sup>130</sup> In contrast, Eady *et al.* could show that the therapeutic control of acne was lost when *P. acnes* developed resistance to erythromycin.<sup>131</sup> The therapeutic control could be regained when an antibiotic was used against which these bacteria were still sensitive. *In vitro* experiments revealed that cell wall extracts as well as exocellular lipase of *P. acnes* are potent chemoattractants for leukocytes, like

neutrophils.<sup>132</sup> Therefore, these bacteria may have an important role in the promotion of inflammatory reactions *in vivo*. On the other side PDT of acne vulgaris with topical 5-aminolaevulinic acid showed an apparent improvement of facial appearance and a reduction in the development of new acne lesions.<sup>133</sup> Recently published reports indicate a selective damage to sebaceous glands, hair follicles and epidermis.<sup>133,134</sup> After recovery a normal skin structure is maintained except for a persistent reduction in the number of hair follicles (decreased number of pilosebaceous units). Therefore PDT could be beneficial in the treatment of acne not only by cytotoxic effects of the skin but perhaps PDT also has antibacterial effects against *Propionibacterium ssp.*<sup>135</sup>

## Conclusions

From its early beginnings antibacterial photodynamic therapy has more and more become an alternative therapeutic approach for the treatment of microbial induced skin diseases. Primarily, antibacterial photodynamic therapy was used for the disinfection of whole blood and blood products. The listed advantages (see above) suggest the use of antibacterial PDT for localized bacterial or parasitic infections, *e.g.* atopic dermatitis, within the next few years. For the moment, the most promising feature is the local application of a photosensitizer and the subsequent localised reactions, thereby not harming the surrounding tissue or disturbing the residual flora of the tissue. For this purpose it is indispensable to find a therapeutic window *in vivo* where bacteria and not keratinocytes are killed. Recently, a new technique was developed to kill bacteria without the potential of non-specific damage to normal tissues mediated by a combination of a specific antibody and the photodynamic activity of a photosensitizer. Antibody-targeted photolysis of bacteria *in vivo* against *Pseudomonas aeruginosa* resulted in a >75% decrease in the number of viable bacteria treated with a specific antibody-conjugate, whereas normal bacteria growth was detected after treatment with a non-specific conjugate or untreated.<sup>136</sup> The advantage of antibody-targeted photolysis will be the specificity of antibodies against bacteria together with no toxicity against surrounding tissues. However, the high molecular weight of such complexes will inhibit penetration into the skin. From this point of view the development of methods to enhance the penetration of topical PS through the epidermal permeability barrier is of importance.

The development of bacterial resistance to photosensitization is still under debate. Resistance may occur *via* different mechanisms. First of all gram (–) bacteria could reduce or prevent the uptake of the PS through the outer membrane. Thus a reduced binding of PS to the cytoplasmic membrane is possible and subsequently a reduced killing of these bacteria. Ehrenberg *et al.* have shown by fluorometric studies that the outer membrane and cell wall shield the cytoplasmic membrane from binding of porphyrins and porphyrin-like molecules.<sup>40</sup> However, to reach the same extent of logarithmic decrease in cell survival, a three times longer illumination is necessary to kill *E. coli* in contrast to gram (+) bacteria.<sup>15,16,36</sup> Nevertheless, cationic phthalocyanine and porphyrin photosensitizers are capable to reduce the cell survival both of gram (+) and gram (–) microorganisms. The uptake of these compounds by gram (–) bacteria is mediated *via* the so called self-promoted uptake pathway. A reduced or prevented uptake through this pathway may be explained by an overall reduced negative charge of LPS-molecules, thus a decreased amount of dyes could bind to the LPS. This possible mechanism was shown by an isolated strain of *Salmonella enterica* serovar Typhimurium which had resistance to polymyxin B, a polycationic compound.<sup>137</sup> In addition, this strain encodes a protein that is responsible for the resistance against polymyxin B. This protein pmrD is part of the component regulatory system to modify lipid A in the outer membrane.<sup>138</sup> Another possible mechanism of resistance of gram (–) bacteria against photosensitization may result

from the resistance against antibiotics. Porin-deficient mutants, resistant to certain beta-lactam molecules, were first isolated from laboratory strains, e.g. *E. coli* K12.<sup>139</sup> As a consequence this strain lacked the general (non specific) porin OmpF, thus reducing the penetration of unspecific compounds.

Resistance of gram (+) bacteria to antibiotics and biocides due to a restricted penetration can occur, as shown by vancomycin-intermediate resistant *Staphylococcus aureus* (VISA) strains which produce a markedly thicker peptidoglycan layer.<sup>140,141</sup> Thus, cell wall changes such as an increased thickness of the cell wall or different cross-linking patterns of the peptidoglycan layer would lead to a decreased penetration of PS. Whether bacteria could develop resistance to reactive oxygen species, e.g. singlet oxygen is questionable. Up to now, there has existed no report, concerning a potential specific resistance mechanism against reactive oxygen species. In general, the development of resistance to photosensitization by microbial strains should be considered as an unlikely event since this process is typically multi-target, which is at variance with the mechanism of action of most antibiotics.

Topical application of antibacterial PS needs an appropriate formulation to reach bacteria interepidermally. Therefore effects of formulations, including cream, emulsion, lotion, nanocolloid and ointment are essential on the penetration and/or accumulation of these PS and demand further investigations.

In summary, the formulation, pharmacokinetics, and the type of PS, the duration between its administration and light application and the region or extent of body surface area exposed to activating light may influence the impact of PDT on microorganisms relevant in dermatologic diseases.

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