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# Fast and effective: intense pulse light photodynamic inactivation of bacteria

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**Abstract** The goal of this study was to investigate the photodynamic toxicity of TMPyP (5, 10, 15, 20-Tetrakis (1-methylpyridinium-4-yl)-porphyrin tetra *p*-toluenesulfonate) in combination with short pulses (ms) of an intense pulse light source within 10 s against *Bacillus atrophaeus*, *Staphylococcus aureus*, Methicillin-resistant *S. aureus* and *Escherichia coli*, major pathogens in food industry and in health care, respectively. Bacteria were incubated with a photoactive dye (TMPyP) that is subsequently irradiated with visible light flashes of 100 ms to induce oxidative damage immediately by generation of reactive oxygen species like singlet oxygen. A photodynamic killing efficacy of up to 6 $\log_{10}$ (>99.9999%) was achieved within a total treatment time of 10 s using a concentration range of 1–100 µmol TMPyP and multiple light flashes of 100 ms (from 20 J $cm^{-2}$ up to 80 J $cm^{-2}$). Both incubation of bacteria with TMPyP alone or application of light flashes only did not have any negative effect on bacteria survival. Here we could demonstrate for the first time that the combination of TMPyP as the respective photosensitizer and a light flash of 100 ms of an intense pulsed light source is enough to generate sufficient amounts of reactive oxygen species to kill these pathogens within a few seconds. Increasing antibiotic resistance requires fast and efficient new approaches to kill bacteria, therefore the photodynamic process seems to be a promising tool for disinfection of horizontal surfaces in industry and clinical purposes where savings in time is a critical point to achieve efficient inactivation of microorganisms.

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## Introduction

### Sterilization processes

Successful inactivation or sterilization of pathogenic microorganisms is one important goal in a world of increasing multi-resistant pathogens in industry and medicine [3, 4, 9, 33]. Autoclaving (121°C steam) and thermal sterilization (hot air 220°C only) can not be used for some polymeric surface materials because of the high temperature used. The use of UV and $\gamma$-radiation is dangerous and critical because it can damage the DNA structure and induce mutagenesis [14]. Chemical agents such as ethylene oxide or hydrogen peroxide for sterilization are limited because of their toxicity to eukaryotic cells and the high amounts of fresh water needed for rinsing after application [26, 43]. Furthermore, chlorination is a widely used technique for disinfection, but the environmental impact of chlorine itself can be problematical [15, 45]. Over the last decades, silver, in terms of silver cations, was introduced as a disinfectant for the treatment of water, in dietary supplements, in medical applications, which can produce antimicrobial coatings and products [42].

### Photodynamic antimicrobial action

Recently, photodynamic treatment of microorganisms has been shown to be very effective in vitro as well as in vivo [17–19, 29, 32, 38]. Photodynamic inactivation of microorganisms is based on the concept that positive-charged photosensitizers can attach and/or accumulate in or at the

pathogen to induce irreversible damage upon light activation of the photosensitizer [2, 36]. The absorption of light by a photosensitizer leads to the generation of reactive oxygen species such as singlet oxygen, which induces an irreversible oxidative damage of the pathogens during illumination [30]. The presence of multiple positive charges enables the photosensitizer agent to interact with the negatively charged outer cell wall areas of bacteria, in particular with the negatively charged lipopolysaccharides of Gram-negative bacteria [34]. Tetra-cationic porphyrins are very effective against bacteria, like TMPyP (5, 10, 15, 20-Tetrakis (1-methylpyridinium-4-yl)-porphyrin tetra *p*-toluenesulfonate) [40]. An incubation of 5 min with TMPyP and very long illumination times in the range of 1–30 min with 60 mW $cm^{-2}$ using an incoherent light source achieved a killing efficacy of >5 $log_{10}$, corresponding to a total radiant exposure of 3.6–108 J $cm^{-2}$ [16]. These results strengthen the research interest for optimization of the photodynamic process regarding incubation time and applied radiant exposure using standard new incoherent light devices, to get overall treatment times within a few seconds.

### Incoherent light sources

Many light sources have been applied and approved for topical photodynamic tumor treatment in dermatology, such as lasers and incoherent light sources [11]. Incoherent light sources have the advantage of a broad emission spectrum (400–1,000 nm) in the visible wavelength range, which covers the absorption spectra of many photosensitizers. Within these devices, intense pulsed light sources (IPLs) have been developed for different dermatological procedures and treatments, for instance rejuvenation of photo-damaged skin, removal of port-wine stains, and aesthetic challenges such as hair removal [20, 39, 41]. IPLs are high-power flash lamps that can emit millisecond pulses at high radiant emittance (mW $cm^{-2}$). The basic principle of an IPL is selective thermal damage of the tissue target depending on the wavelength spectrum, pulse duration, and fluence. Short pulses reduce the diffusion of heat in deeper areas inside the tissue to avoid by-effects. The wavelength range of an IPL applicator is blocked by an optical edge filter, called the cut-off filter.

### Aim of the study

By using such short IPL light pulses and short incubation times of a few seconds, we aimed to achieve a fast and effective photodynamic inactivation of bacteria yielding more than 3 $log_{10}$ (>99.9%). *Staphylococcus aureus*, methicillin-resistant *S. aureus* (MRSA), and *E. coli*, prevalent pathogens of serious concern in hospital-acquired infections, were the principal bacteria included in this study [8]. Furthermore, for industrial purposes, *Bacillus atrophaeus* was tested regarding its sensitivity to the photodynamic process.

## Materials and methods

### Bacterial strains

Biochemical analysis and resistance testing of each bacterial strain were done with a VITEK 2 system (bioMérieux, Nuertingen, Germany) according to the guidelines of the National Committee for Clinical Laboratory Standards (NCCLS, Wayne, PA). Methicillin-sensitive *S. aureus* (MSSA; ATCC 25923), MRSA (ATCC BAA-44 and ATCC 43300), *B. atrophaeus* (ATCC 9372), and *E. coli* (ATCC 25922) were used, which were grown aerobically at 37°C in Mueller–Hinton broth (Gibco Life Technologies GmbH, Eggenstein, Germany). An overnight bacteria culture (5 ml) was harvested by centrifugation (200 × *g*, 15 min), washed with 0.01 mol $l^{-1}$ mM phosphate-buffered saline (PBS; Biochrom, Berlin, Germany) at pH 7.4 containing 0.027 mol $l^{-1}$ KCl and 0.14 mol $l^{-1}$ NaCl, and suspended in PBS at an optical density of 0.6 at 600 nm, which corresponded to $\sim 10^{7-8}$ bacteria $ml^{-1}$, for use in the phototoxicity experiments.

### Light source and irradiation parameters

A commercially available IPL (VL-2 Applicator; Ellipse A/S, Hørsholm, Denmark) was used in this study with the following nominative cut-off excitation filter set of 550 nm (Fig. 1a). The manufacturer's settings for pulses are 100 ms or 83 ms corresponding to 20 or 10 J $cm^{-2}$, respectively. Multiple pulses were applied to establish dose–response curves as follows: (I) 1 × 20 J $cm^{-2}$ followed by increments of 20 up to 80 J $cm^{-2}$, pulse duration was 100 ms. (II) 1 × 10 J $cm^{-2}$ followed by increments of 10 up to 40 J $cm^{-2}$, pulse duration 83 ms. Suspensions of bacteria were incubated with a photosensitizer and subsequently irradiated. Illumination was done from the bottom side of the 96-well plates to avoid refraction of the light in the cell culture media. The optical power of such ILP is in the range of Watts, requiring an exposure time of milliseconds to achieve an effective radiant exposure (J $cm^{-2}$) (see Eq. 1):

$$\text{radiant exposure} \quad \left[\frac{\text{J}}{\text{cm}^2}\right] = \frac{\text{power}}{\text{area}} \times \text{time} \quad \left[\frac{\text{W}}{\text{cm}^2} \times \text{s}\right] \tag{1}$$

The emission spectra of the IPL were normalized to it corresponding to maxima between 750 and 850 nm as previously described [31].

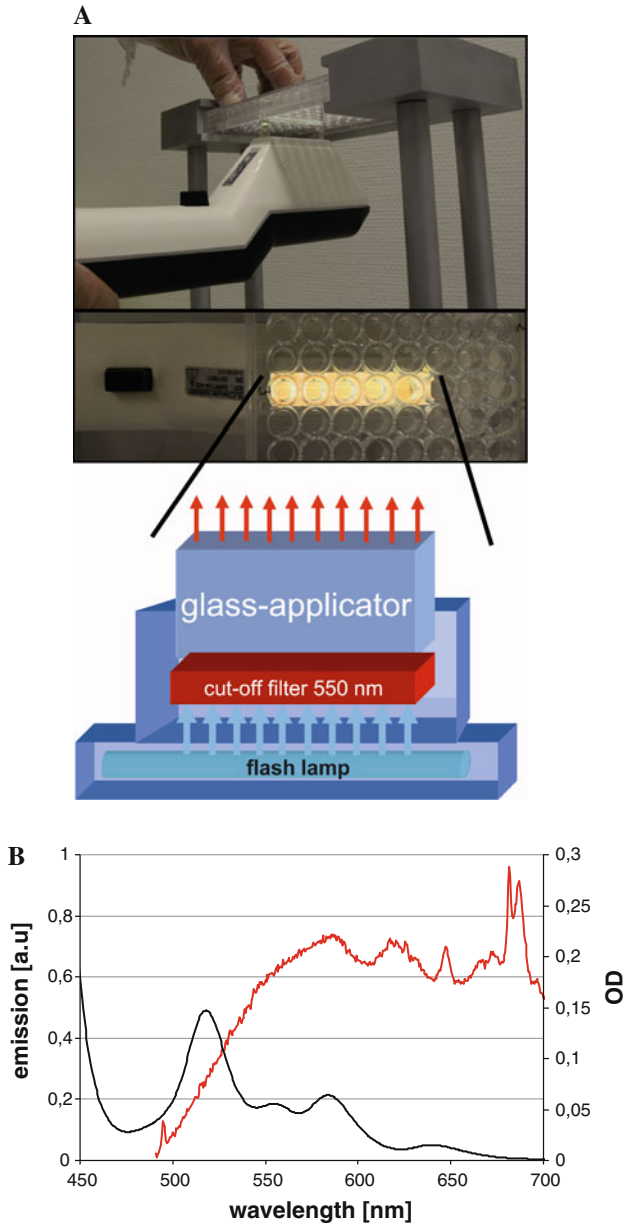


**Fig. 1** IPL applicator and spectral emission of the IPL and absorption spectra of TMPyP. **a** IPL-handheld containing a glass applicator in action (*upper part*) illuminating five wells of a 96-well plate (*middle part*). Schematic drawing of an IPL-handheld (*lower part*). **b** Absorption spectrum of 10 μmol $l^{-1}$ TMPyP (*black*) is shown in the range between 450 and 750 nm (Q-bands I–IV) and the emission spectra of the IPL in the same range between 500–700 nm (*red*). The emission wavelength data of the IPL was normalized to their corresponding maxima between 750 and 850 nm, which is not shown here. *OD* optical density (color figure online)

## Detection of singlet oxygen

The ability of TMPyP to generate singlet oxygen was qualitatively evaluated using a frequency-doubled Nd:YAG laser (PhotonEnergy, Ottensoos, Germany), as previously described, at a wavelength of 532 nm [30, 31]. Singlet-oxygen luminescence was detected with an IR-sensitive photomultiplier (R55-09-42, Hamamatsu Photonics Deutschlang GmbH, Herrsching, Germany) at different wavelengths from 1,200 to 1,350 nm using a monochromator (HORIBA Jobin Yvon Inc. Kyoto, Japan) in front of the multiplier generated by 50 μmol $l^{-1}$ of TMPyP in $H_2O$.

## Photosensitizer TMPyP

TMPyP was purchased from Sigma-Aldrich (Taufkirchen, Germany), purity 97%. TMPyP was dissolved in distilled water at a stock concentration of 0.002 mol $l^{-1}$, passed through a 0.22-μm pore-size filter and stored at 4°C until use. Dilutions were done in PBS-buffer (PAA Laboratories GmbH, Pasching, Austria). The absorption spectrum of 100 μmol $l^{-1}$ TMPyP was measured in aqua dist.

## Phototoxicity assay of the bacteria

A total of $\sim 10^{7-8}$ bacterial cells per milliliter were placed into a 96-well microtiter plate (100 μl/well) and incubated with different concentrations of TMPyP (0, 1, 10, and 100 μmol $l^{-1}$) for 10 s in the dark at room temperature. Immediately at the end of the incubation period, the bacteria were illuminated with the IPL. Controls were neither sensitized with TMPyP nor exposed to the light source or were incubated with a photosensitizer only or illuminated only. After illumination, the survival of the bacteria was determined by counting the number of CFU using the Miles, Misra and Irwin technique [35]. Serially diluted aliquots of treated and untreated (no photosensitizer, no light) cells were plated on Mueller–Hinton agar, and the number of CFU per milliliter was counted after 24 h of incubation at 37°C.

## Statistical methods

All results are shown as medians, including the 25 and 75% quartiles, which were calculated from the values of at least three independent experiments. Each experiment was conducted in triplicate, with Prism 4 for Windows (GraphPad Software Inc., San Diego, CA, USA). The calculation was referred to untreated controls (bacteria, but no light and no photosensitizer) (black horizontal line). In Figs. 3, 4 and 5, medians on or below the dotted horizontal lines represent ≥99.9% efficacy or ≥99.999% of bacteria killing, corresponding to at least more than three magnitudes or five magnitudes of $\log_{10}$ reduction compared to matching untreated controls. A reduction of at least three magnitudes of $\log_{10}$ of viable median numbers of bacteria was stated as biologically relevant with regard to the

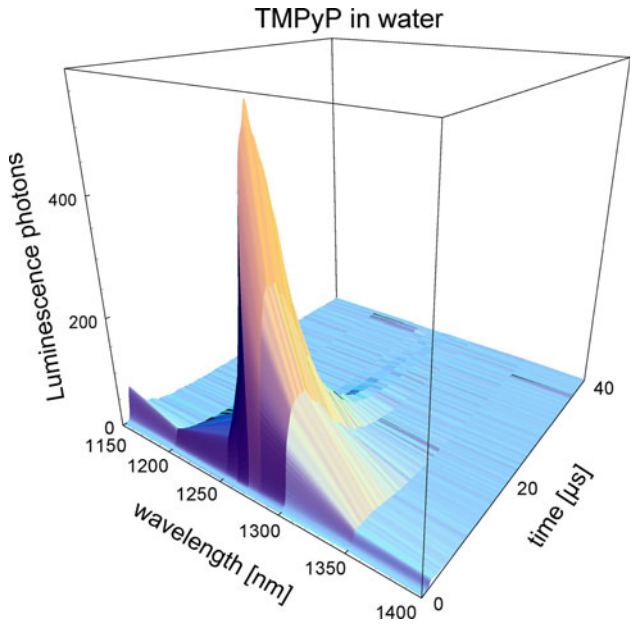


**Fig. 2** Fingerprint of singlet oxygen generation by TMPyP. Wavelength scan by summing up the luminescence signals of singlet oxygen at different wavelengths from 1,170 to 1,370 nm generated by 50 µmol $l^{-1}$ TMPyP in $H_2O$

guidelines of hand hygiene [10]. Percentage of phototoxicity was calculated as follows (Eq. 2:

$$\frac{\text{CFU control} - \text{CFU sample}}{\text{CFU control}} \times 100 = \quad \% \text{ of reduction} \tag{2}$$

## Results

### Overlap of the TMPyP absorption spectrum with the emission spectra of the IPL device

Generally, light absorption by TMPyP for wavelengths above 700 nm is very low and no emission wavelengths of the IPL were detected below 490 nm. Therefore, the spectral overlap of TMPyP absorption and the IPL emission was considered in the range of 490–750 nm. Figure 1 shows the extent of the spectral overlap of the respective IPL and TMPyP. The emission spectrum of the IPL closely matches the four absorption peaks of TMPyP, the so-called Q-bands at 517, 554, 584, and 640 nm (Fig. 1).

### Singlet oxygen generation and detection

The singlet oxygen generation by TMPyP upon excitation with visible light is shown in Fig. 2. The combination of the spectral and time-resolved distribution of the singlet-oxygen luminescence signals detected by the laser/photomultiplier-system confirms the generation of singlet oxygen by TMPyP resulting in a characteristic fingerprint of singlet oxygen, which is in good agreement with already published data for singlet oxygen in aqueous solutions [30].

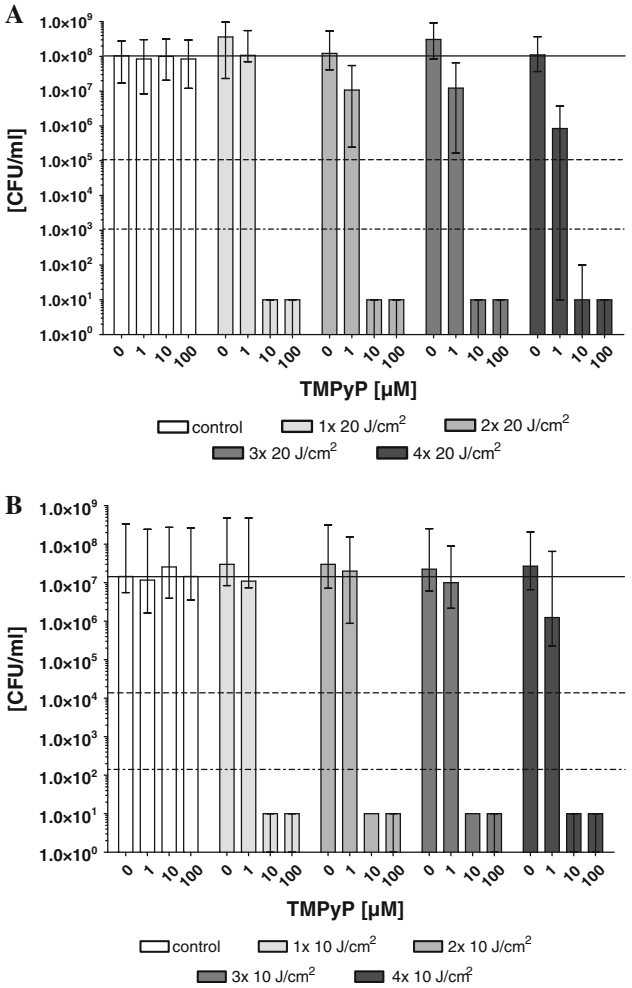


**Fig. 3** Photosensitized inactivation of MSSA *S. aureus*. Survival of MSSA incubated with different TMPyP concentrations for 10s in the dark and followed by illumination with **a** multiple light flashes of 100 ms ($1 \times 20$ J $cm^{-2}$ up to $4 \times 20$ J $cm^{-2}$) or **b** 83 ms (10 J $cm^{-2}$ each). Control (*white bars*): bacteria alone or incubated with TMPyP only, but not irradiated. *Bars* represent the median, including the 25 and 75% quartiles, of three independent experiments. Values on or below the *dotted horizontal line* represent ≥99.9% or ≥99.999% (*chain-dotted line*) efficacy of bacteria killing, which was referred to the untreated controls (bacteria alone, no light, no photosensitizer)

### IPL induced phototoxicity of TMPyP-sensitized MSSA *S. aureus* and *E. coli*

Different clinical and industrial pathogens were incubated with different concentrations of TMPyP for 10 s only. Immediately after incubation, the bacteria were illuminated with different light flashes, which correspond to applied radiant exposures of 10 up to 80 J $cm^{-2}$. Incubation of MSSA with TMPyP caused a biologically relevant decrease

in CFU/ml upon illumination with multiple light flashes (Fig. 3a, b). A TMPyP concentration of 1 μmol $l^{-1}$ already exhibited a killing efficacy of ~2 magnitudes of $\log_{10}$ reduction at a radiant exposure of 80 J $cm^{-2}$ (4× flashes, 20 J $cm^{-2}$ each). Incubation with higher concentrations of TMPyP (10 or 100 μmol $l^{-1}$) showed a further decrease in bacterial survival of ≥5 $\log_{10}$ orders (killing efficacy of 99.999%) using only one light flash of 20 J $cm^{-2}$ (Fig. 3a). Antibacterial activity (≥99.999% killing efficacy) seemed to plateau with increasing radiant exposure (up to 80 J $cm^{-2}$). In addition, MSSA was already killed to more than 99.999% at 10 μmol $l^{-1}$ of TMPyP when the radiant exposure was reduced to 10 J $cm^{-2}$ (Fig. 3b).

Illumination with multiple radiant exposure of 20 J $cm^{-2}$ of TMPyP-sensitized *E. coli* revealed a smaller decrease in CFU/ml compared to the killing efficacy of MSSA (Fig. 3 vs. Fig. 4). There was a biologically relevant decrease of 5 $\log_{10}$ (≥99.999% reduction) in CFU/ml of *E. coli* when irradiated with 40 J $cm^{-2}$ (2 × 20 J $cm^{-2}$) after photosensitization with 100 μmol $l^{-1}$ TMPyP compared to the control group (Fig. 4a). However, a radiant exposure of 20 J $cm^{-2}$ exhibited a biologically relevant killing efficacy of 3 $\log_{10}$ (99.9%) only at a used TMPyP concentration of 100 μmol $l^{-1}$. TMPyP concentrations of less than 100 μmol $l^{-1}$ did not induce a biologically relevant photodynamic inactivation regardless of the used radiant exposure of up to 80 J $cm^{-2}$ (Fig. 4a). Furthermore, Fig. 4b showed the killing efficacy within 10s of TMPyP at 100 μmol $l^{-1}$ against *E. coli* depending on the radiant exposure: ~1 magnitudes of $\log_{10}$ reduction after one flash, ~3 magnitudes of $\log_{10}$ reduction after two flashes, >3 magnitudes of $\log_{10}$ reduction after three flashes and >5 magnitudes of $\log_{10}$ reduction after four flashes, which correspond to radiant exposure of 10, 20, 30, and 40 J $cm^{-2}$, respectively (Fig. 4b).

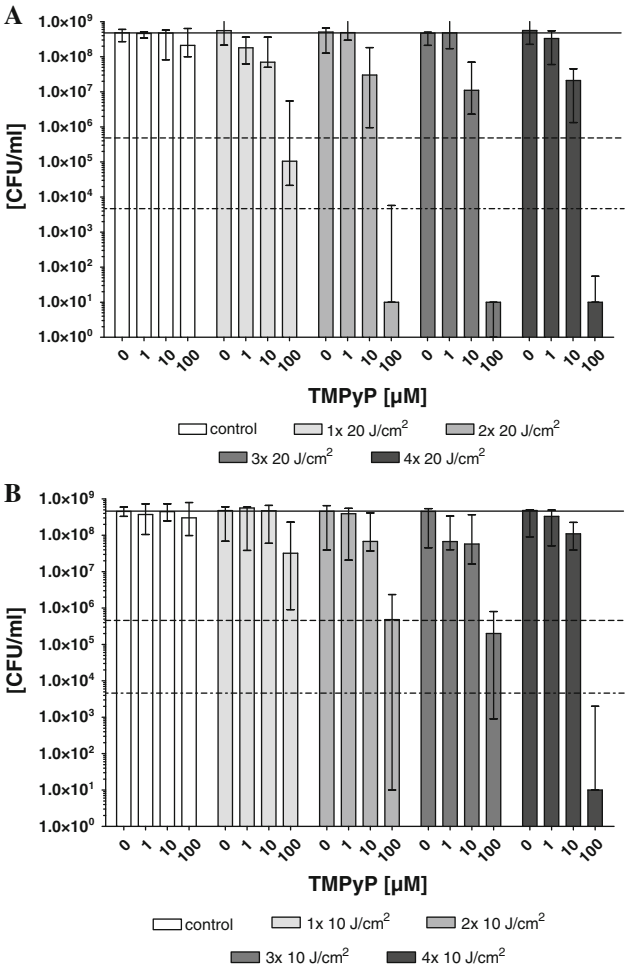


**Fig. 4** Photosensitized inactivation of *E. coli*. Survival of *E. coli* incubated with different TMPyP concentrations for 10 s in the dark and followed by illumination with **a** multiple light flashes of 100 ms (1 × 20 J $cm^{-2}$ up to 4 × 20 J $cm^{-2}$) or **b** 83 ms (10 J $cm^{-2}$ each). Control (*white bars*): bacteria alone or incubated with TMPyP only, but not irradiated. *Bars* represent the median, including the 25 and 75% quartiles, of three independent experiments. Values on or below the *dotted horizontal line* represent ≥99.9% or ≥99.999% (*chain-dotted line*) efficacy of bacteria killing, which was referred to the untreated controls (bacteria alone, no light, no photosensitizer)

## IPL induced phototoxicity of TMPyP-sensitized MRSA and *B. atrophaeus*

In order to investigate whether the observed growth reduction of methicillin-sensitive *S. aureus* (MSSA) was independent of the antibiotic resistance pattern, two MRSA strains were photosensitized with TMPyP for 10 s and illuminated under conditions identical to those used for the MSSA strain. In repeated experiments, both MRSA strains showed a similar decrease in CFU/ml as the MSSA strain (Table 1). TMPyP at a concentration of 10 μmol $l^{-1}$ achieved a substantial killing efficacy of 5 $\log_{10}$ already after a light flash of 10 J $cm^{-2}$ against both MRSA strains tested. As shown in (Table 1) higher applied radiant exposures up to 80 J $cm^{-2}$ did not further increase the killing efficacy compared to the growth of the controls without illumination. In addition, *B. atrophaeus* was already killed to more than 4 $\log_{10}$ at 10 μmol $l^{-1}$ of TMPyP and only one light flash of 10 or 20 J $cm^{-2}$ (Fig. 5). Again increasing the radiant exposure seemed to plateau the killing efficacy of *B. atrophaeus*.

Overall, all bacterial samples that were incubated without photosensitizer exhibited unaffected growth with and without illumination, demonstrating that the maximal radiant exposure of 80 J $cm^{-2}$ at the level of the illuminated bacteria samples has no antibacterial effects (Figs. 3, 4 and 5).

## Discussion

Various studies have shown that photodynamic inactivation of bacteria with light in the visible wavelength range and

**Table 1** Phototoxicity of different radiant exposures against TMPyP-sensitized MRSA strains BAA-44 and 43300

| TMPyP | $Log_{10}$ reduction of viable bacteria number depending on the radiant exposure | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 83-ms pulse duration | | | | | | | | 100-ms pulse duration | | | | | | | |
| | 10 J $cm^{-2}$ | | 20 J $cm^{-2}$ | | 30 J $cm^{-2}$ | | 40 J $cm^{-2}$ | | 20 J $cm^{-2}$ | | 40 J $cm^{-2}$ | | 60 J $cm^{-2}$ | | 80 J $cm^{-2}$ | |
| 1 μM | 0.65[a] | 0.23[b] | 1.9[a] | 1.97[b] | 0.98[a] | 2.99[b] | 0.9[a] | 2.99[b] | 0.75[a] | 2.96[b] | 2.98[a] | 2.99[b] | 2.99[a] | 3[b] | 3[a] | 3[b] |
| 10 μM | 4 | 3.9 | 4 | 3.9 | 4 | 3.9 | 5 | 5 | 4 | 5 | 5 | 5 | 5 | 5 | 5 | 5 |
| 100 μM | 5 | 3.9 | 5 | 3.9 | 5 | 3.9 | 5 | 5 | 5 | 4 | 5 | 5 | 5 | 5 | 5 | 5 |

[a] MRSA, strain BAA-44

[b] MRSA, strain 43300

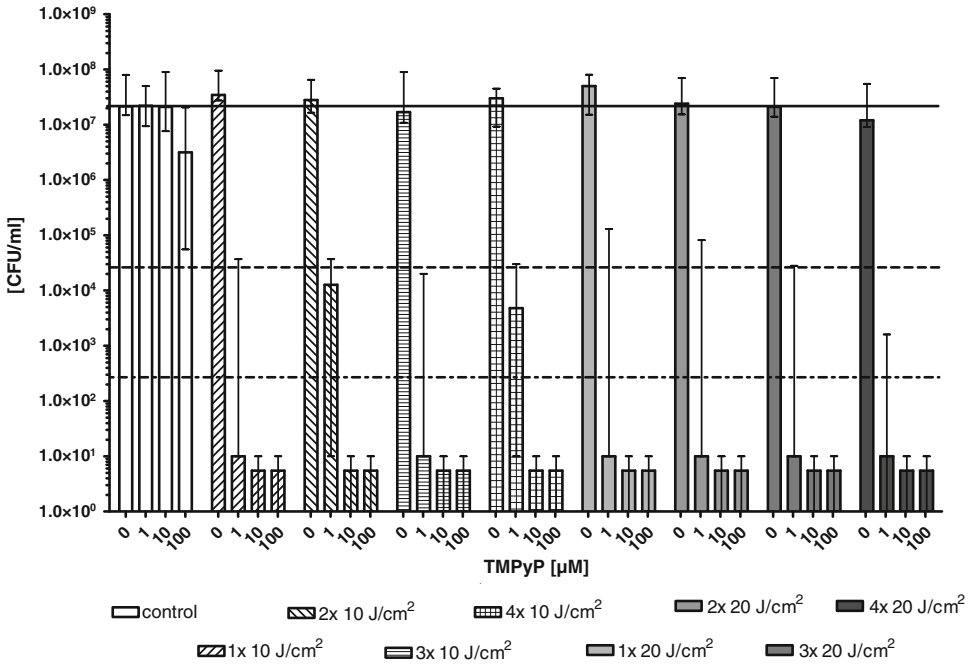


**Fig. 5** Photosensitized inactivation of *B. atrophaeus*. Survival of *B. atrophaeus* incubated with different TMPyP concentrations for 10 s in the dark and followed by illumination with multiple light flashes of 83 ms (*grey bars* crosshatched; 10 J $cm^{-2}$ each) or 100 ms (*grey bars*; 1 × 20 J $cm^{-2}$ up to 4 × 20 J $cm^{-2}$). Control (*white bars*): bacteria alone or incubated with TMPyP only, but not irradiated. *Bars* represent the median, including the 25 and 75% quartiles, of three independent experiments. *Values on or below the dotted horizontal line* represent ≥99.9 or ≥99.999% (*chain-dotted line*) efficacy of bacteria killing, which was referred to the untreated controls (bacteria alone, no light, no photosensitizer)

porphyrin-based photosensitizers, like TMPyP, exhibit significant photosensitizing activities against a broad range of pathogens only in the presence of light and oxygen, but takes overall very long treatment times due to the low intensity of the available light sources [28, 32]. In accordance with already published data, here in this study a characteristic fingerprint of singlet oxygen was detected upon excitation of TMPyP at 532 nm [30]. Furthermore, the quantum yield of singlet oxygen of TMPyP is ~70%, which is high compared to other known photosensitizers [13, 21, 46] like Methylene blue (52%) [44] or Photofrin (36%) [37].

The results of the present study showed that a commercially available IPL device with a standard cut-off filter (550 nm) in combination with TMPyP, as the respective photosensitizer, is able to inactive different pathogens very fast and effective upon illumination with visible light. A very short incubation time of 10 s in combination with a low photosensitizer concentration of 10 μM and a short light exposure time of 100 ms was effective in killing methicillin-sensitive *S. aureus*, two different MRSA strains, *E. coli* and *B. atrophaeus* with an efficacy of 99.999% (5 $log_{10}$ reduction).

A commercially available IPL was used as a light source, which has become increasingly popular for several reasons [5]. (1) IPLs were developed for different dermatological conditions including photodynamic therapy

(PDT) and, therefore, IPLs are commonly used in dermatological practice. (2) IPLs can emit red light that overlaps with the absorption peaks of the Q-bands of porphyrin photosensitizers such as TMPyP. Therefore the use of such an IPL for antimicrobial photodynamic treatment would considerably shorten treatment times up to a few seconds in comparison to LED systems or other incoherent light sources, which take at least about 5–30 min depending on the extent of radiant exposure [27].

IPLs are high-power flash lamps that can emit millisecond pulses at high intensities (hundreds of W $cm^{-2}$). Here no thermal damage of bacteria was observed because irradiation alone did not affect viability as compared to untreated controls (no light and no photosensitizer). Thus, bacteria killing was based solely on photodynamic action. Moreover, it is likewise of importance that the emission spectrum of the used IPL applicator sufficiently match the absorption spectra of TMPyP to achieve maximum antimicrobial killing efficacy. In our study, the Q-bands of TMPyP effectively absorb the emitted visible light of the IPL applicator (Fig. 1b). Such a cut of filter of 550 nm was selected, because only pure visible light should be used for this study. Due to safety reasons, UVA radiation was completely excluded concerning protection of the operator and the environment. When the Soret band of TMPyP should be light-activated, a suitable longpass-filter notation would let pass as well part of the UV-A radiation due to the transmission range of the filter. The results of this study clearly showed that it is not necessary to include the wavelength range for excitation of the Soret band at 420 nm of TMPyP for an enhanced light activation because photodynamic inactivation efficacy was extremely sufficient (>5 $\log_{10}$ steps).

Therefore, the short light flashes of 83 or 100 ms were sufficient to start the photodynamic process using TMPyP, which generated reactive oxygen species such as singlet oxygen via type-II mechanism [27]. Therefore, the generation of singlet oxygen by IPL excited TMPyP is highly efficient and the interaction of singlet oxygen with the energy-producing membrane area systems of bacteria leads to a dissipation of the membrane potential [28].

Since the dramatic worldwide increase of antibiotic resistance both inside and outside of health care settings, new strategies to control infection are of interest [12]. At present, a wide variety of antibiotics, antiseptics, and disinfectants is in use where bacteria achieve multiple drug resistance [1, 6, 7]. Such a multiple drug resistance is spread within the bacterial community and this situation is leading to pathogens potentially resistant to any available antibiotic [25]. In general photodynamic killing efficacy was not influenced using a porphyrin-based photosensitizer against either a fluoroquinolon-resistant *E. coli* strain (efflux pump over-expression) or its wild-type. The corresponding data are not presented due to not having differences in photodynamic inactivation efficacy.

So far, no bacteria strain is known that has developed resistance to the photodynamic process. Grinholc et al. could demonstrate that various porphyrin-based photosensitizers tested executed their antibacterial activity with no change in the antibiotic resistance pattern of the studied MRSA strains [23]. So far only the bactericidal effect of photodynamic inactivation against methicillin-resistant and methicillin-susceptible *S. aureus* is strain-dependent, which means that different photodynamic parameters (applied radiant exposure, concentration, and incubation time) are necessary to achieve a complete eradication of different pathogens tested [22]. Furthermore, the strain-dependent differences of bactericidal photodynamic inactivation could not be correlated to either the levels of photosensitizer uptake or the pharmacological inhibition of the efflux pump [24]. Therefore, other factors might be influencing such observed differences in the photodynamic efficacy, such as aggregation of the photosensitizer, concentration of antioxidant enzymes, or cellular repair proteins, but all of these factors are not responsible for diminishing TMPyP-induced photosensitization because up to now, there is a lack of selection of photo-resistant bacteria after multiple photodynamic treatments [27]. Furthermore, the advantage of such a very short photodynamic treatment time of seconds is that it might not allow bacteria to react to the photodynamic process to attain resistance. Here, the photodynamic inactivation of *E. coli* indicates that stronger conditions were necessary than for the Gram-positive bacteria. According to the composition of the cell wall of Gram-positive and -negative bacteria, different porphyrine derivatives demonstrate variable degrees of bactericidal efficacy upon illumination. The presence of the outer membrane makes Gram-negative bacteria more resistant to photodynamic inactivation than Gram-positive bacteria [36]. Gram-negative bacteria, such as *E.* coli, possess an outer membrane, located outside the peptidoglycan layer consisting of negatively charged lipopolysaccharides (LPS) and some major proteins that compound the uptake of PS.

Besides medical application of IPL-based antimicrobial technology, this procedure should also be feasible in environmental technology. Considering safety, the use of light-activating molecules is safe due to the used wavelengths being part of the visible light spectrum and coupled with the fact that photodynamic inactivation occurs only when the light is switched on does not require additional chemical pre-treatments. The photodynamic process for microbial inactivation makes it very attractive for a range of potential decontamination applications, like cleaning and disinfection of horizontal surfaces and items in both

health-care settings and industry, where liquid disinfection like alcohol, perchlorate, or $H_2O_2$ is not possible because protection is needed of corrosive material.

Overall, the results of this study clearly demonstrate for the first time that an IPL device is suitable in combination with TMPyP to induce a photodynamic process to inactivate clinical or industrial pathogens efficiently within a few seconds only. It should be emphasized that TMPyP has no clinical approval to be used as a medical drug. However, these results should encourage the development of similar porphyrin molecules with similar properties that can be approved in the near future. The rapid inactivation procedure within seconds suggest that it may be useful as a promising tool for industrial and clinical purposes where savings in time is a critical point to achieve efficient inactivation of microorganisms.

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