Systematic Review

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Antimicrobial efficacy of irradiation with visible light on oral bacteria *in vitro*: a systematic review

Aim: Resistances to antibiotics employed for treatment of infectious diseases have increased to alarming numbers making it more and more difficult to treat diseases caused by microorganisms resistant to common antibiotics. Consequently, novel methods for successful inactivation of pathogens are required. In this instance, one alternative could be application of light for treatment of topical infections. Antimicrobial properties of UV light are well documented, but due to its DNA-damaging properties use for medical purposes is limited. In contrast, irradiation with visible light may be more promising. **Method:** Literature was systematically screened for research concerning inactivation of main oral bacterial species by means of visible light. **Results:** Inactivation of bacterial species, especially pigmented ones, in planktonic state showed promising results. There is a lack of research examining the situation when organized as biofilms. **Conclusion:** More research concerning situation in a biofilm state is required.

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Far back in 1945, Sir Alexander Fleming already mentioned in his noble prize speech that there may be the ability of bacteria to get resistant to antibiotics [1]. Since then, antimicrobial resistances of microorganisms have become an increasing problem to public health making it more difficult to treat diseases caused by resistant pathogens in lack of proper treatment modalities. This can lead to severe infections or even death of patients [2]. In recent years, it has been reported that some bacterial strains have become resistant to all available antibiotics [3]. Thus, development of novel strategies for treatment of infections caused by antibiotic-resistant pathogens has to be a major research goal in life sciences. Also in the field of dentistry, administration of systemic and topic antibiotics should be reduced as much as possible for reducing the risk of inducing new resistances. Furthermore, there are more and more refractory infections of endodontic and periodontal origin caused by antibiotic-resistant pathogens [4,5].

In this instance, the antimicrobial photodynamic therapy (aPDT) may be a promising alternative for topical killing of bacteria [6,7]. Usually, aPDT consists of application of an external substance, the so-called photosensitizer (PS), and subsequent irradiation with light of an appropriate wavelength, resulting in generation of reactive oxygen species (ROS) that kill bacteria by oxidative processes [8]. Pronounced inactivation rates of oral biofilms have already been shown for aPDT [9].

On the other hand, it is known that some bacteria are sensitive to irradiation with light only without the application of a light-sensitive molecule [10,11]. For instance, irradiation with UV light, whose spectrum is subdivided into three sections (UV-C: 100–280 nm,

UV-B: 280–315 nm and UV-A: 315–380 nm), proved to be able to inactivate bacteria [12]. It was shown that its maximum bactericidal effect occurs within the UV-C range between 240–280 nm [13]. However, there are several disadvantages of using UV light in patients. It has been reported that absorption of UV-A light might result in damage of major biomolecules including DNA and membrane lipids in eukaryotic cells [14]. It is also known that UV light leads to different classes of mutagenic and cytotoxic DNA lesions [15]. High amounts of UV rays can also lead to skin cancer and eye conditions such as cataracts [16]. Thus, the usage of UV light for topical killing of pathogens may not be a proper alternative for treatment of oral infections.

Consequently, application of visible light could be an alternative for inactivation of bacteria. Visible light corresponds to wavelengths between 380 and 750 nm reflecting a color range from violet to red [17]. There are reports suggesting that light from the visible spectrum might lead to an autophotosensitization process inducing production of ROS in pathogens as a result of an accumulation of endogenous substances already present within biofilms or tissue that can act as PS [11]. For control of oral infections, irradiation with visible light may be a favorable approach due to the easy accessibility of the oral cavity compared with other parts of the body.

The aim of this systematic review was to assess the results of studies investigating the efficacy of treatment with visible light without external application of a PS *in vitro* on relevant bacteria occurring in the oral cavity.

Methods

As this systematic review does not study any health-related outcome of direct patient or clinical relevance, it was not considered eligible for registration in the International Prospective Register of Systematic Reviews PROSPERO.

The focused question of this systematic review was: 'Is irradiation with visible light capable of inactivating oral bacteria *in vitro*?'

Identification of studies

Studies were identified by searching electronic databases and scanning the reference lists of eligible articles and relevant reviews. Search strategies were developed by a subject specialist and medical librarian who is trained and experienced in conducting systematic literature searches (H Knüttel). Although we had no opportunity to have the search strategies peer-reviewed, we strived to design, carry out and report the literature search according to current checklists and recommendations [18,19].

In the research question, we identified three search concepts that were combined using the Boolean operator AND: 'photoinactivation,' 'bacteria' and 'oral.' For each of the concepts, search terms including synonyms were compiled and combined using the Boolean operator OR in order to compose a highly sensitive search strategy. We selected feasible search terms, relevant subject headings and appropriate syntax according to the databases and search interfaces. No limits such as for date and language were imposed at the time of searching.

We selected databases by thematic relevance and accessibility. On 22 December 2016, we searched MEDLINE (Ovid: Epub Ahead of Print, In-Process & Other Non-Indexed Citations, Ovid MEDLINE[R] Daily and Ovid MEDLINE[R] 1946 to present), Embase (Ovid: Embase 1974 to 21 December 2016) and Web of Science (Science Citation Index Expanded 1965 to present; data last updated: 21 December 2016). A detailed documentation of the searches allowing for replication is attached in Appendix 1. References were exported from the databases and deduplication was carried out using the method of Bramer *et al.* [20].

The reference lists of eligible articles were scanned independently by two of the authors (subject specialists) for additional relevant articles (A Pummer, F Cieplik). Occasional articles encountered by serendipity in other sources were also included.

Inclusion criteria

In vitro studies examining inactivation of bacteria by means of visible light irradiation without addition of an exogenous light-sensitive substance were taken into consideration. Bacteria could be organized in planktonic state or in biofilm state. Only studies treating bacteria typically occurring in the oral cavity were chosen. Studies published in English or German were included as a consequence of lack of proper expertise in other languages considering scientific issues. Nevertheless, manual screening of different databases leads to the assumption that there were no relevant studies on this topic published in other languages.

Exclusion criteria

Systematic or nonsystematic reviews were excluded. However, eligible studies found in thematically relevant reviews were included. Studies treating other microorganisms than bacteria such as fungi or viruses or studies where exogenous light-sensitive substances were added to bacteria as well as studies in which illumination was performed by means of nonvisible light were not taken into consideration. Non-*in vitro* studies were not taken into consideration as we aimed to show susceptibility of bacteria to light itself like they occur in clinical practice without any outer influences. Moreover, studies examining bacteria that were not of

oral origin or relevance were not included. Conference abstracts without full data or experimental details were excluded.

Data organization

A standard document, which included author(s) and year of publication, investigated microorganisms, organization of microorganisms (planktonic or biofilm), type of light source, irradiation parameters (output power and intensity, wavelength, applied energy) as well as a summary of the main outcomes was used in order to systematize data received from each report (Table 1).

Results & discussion

Study identification

Searching of three electronic databases yielded a total of 4800 records (MEDLINE: 1411; Embase: 2010, Web of Science: 1379). After elimination of duplicates with the Bramer method, 2619 records remained [20]. Eight additional studies were found in the reference lists of the included studies and another four in other sources.

Study selection

In the first step, studies were filtered by title screening by two independent subject specialists (A Pummer, F Cieplik). Studies not relevant by topic were excluded in this step. Second, the abstracts of all of the remaining studies were read and a decision considering suitability was made. In a third step, the full-text articles were read. Only studies that were regarded as suitable after this step were included (Figure 1 for reasons of exclusion).

Screening by title left 262 records that were screened by abstract. 93 articles remained that were screened by reading the full text. Finally, a total of 34 articles relating to the same number of studies (22 articles from the database searches plus 12 studies identified in the reference lists and other sources) were considered as eligible for the review (Figure 1 for a PRISMA [Preferred reporting items for systematic reviews and meta-analyses] flow diagram of the process [54]). Table 1 shows a summary of the 34 studies selected. Table 2 lists a subset of 9 of these 34 studies, where an inactivation of 3 $\log_{10}$ steps (99.9%) or more could be achieved. The microorganism and wavelength tested is listed, as well as the light dose (J/cm^2) required to achieve a 3 $\log_{10}$-step reduction. An inactivation rate of 3 $\log_{10}$ steps was chosen as it is declared as a biologically relevant antimicrobial activity according to infection control guidelines [53].

Actinomyces actinomycetemcomitans

König *et al.* used planktonic suspensions of *Actinomyces odontolyticus* among others and exposed them to red light (60 mW helium–neon laser; 632.8 nm; total energy density: 360 J/cm^2) [44]. The killing rate (CFU [colony forming units] values of laser-exposed bacteria compared with CFU values of nonexposed bacteria) for *A. odontolyticus* was 70 ± 4%, whereas it was 42 ± 10% for *Propionibacterium acnes* and 50 ± 10% for *Porphyromonas gingivalis*. In contrast, no effect could be observed upon illumination of *Streptococcus mutans*. As a result, it can be concluded that there was no antibacterial effect (99.9% reduction or more [53]) for neither of the tested microorganisms. Additional fluorescence measurement proved existence of intracellular protoporphyrin IX for *A. odontolyticus* as well as for *P. acnes* indicating that the observed phototoxicity might be due to an autophotosensitization process [44].

Aggregatibacter spp.

In a study from our group, planktonic suspensions of *Aggregatibacter actinomycetemcomitans* were irradiated with a light-emitting diode (LED) light-curing unit with an emission peak at 460 nm at a total energy dose of 150 J/cm^2 (1250 mW/cm^2; 120 s) [11]. This resulted in a reduction of more than 5 $\log_{10}$ steps (antibacterial effect [53], Table 2) for *A. actinomycetemcomitans*, whereas there was no effect for blue-light irradiation of *Escherichia coli*, which was used as a control organism [11].

Spectroscopic investigations showed presence of intracellular porphyrins and flavins. Excitation at the emission peak of the light source used in the experiments (460 nm) showed that particularly flavins may have been causative for inactivation of *A. actinomycetemcomitans* by irradiation with blue light (Tables 1 & 2) [11].

However, these results are in marked contrast to those of Song *et al.*, who examined the effect of blue-light irradiation on *A. actinomycetemcomitans* in planktonic suspensions as well as in biofilm state [51]. A halogen lamp (λ = 400–520 nm; output power of 500 mW/cm^2) was used for the experiments. Samples were exposed to light for 15–120 s (7–60 J/cm^2). There was no effect for blue-light illumination neither in planktonic nor in biofilm state for *A. actinomycetemcomintans*, whereas blue light was strongly bactericidal to *P. gingivalis* and *Fusobacterium nucleatum* in planktonic state (~100% killing after 15 s of irradiation in the case of *P. gingivalis*, 99.1% following 60 s for *F. nucleatum*, respectively [51]). Effect upon illumination of bacteria organized in biofilm state, however, was statistically significant only for *P. gingivalis* (~1 $\log_{10}$ reduction for 120 s, no antibacterial effect [53]).

As these two studies showed totally different results for phototoxicity of *A. actinomycetemcomitans* toward blue light, no final conclusion on susceptibility of *A. actinomycetemcomitans* to visible light illumination can be drawn so far. The observed differences may be due to different light sources (LED

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Table 1. Summary of all 34 selected studies sorted by authors.

Study (year)	Microorganisms	Wavelength, maximum energy	Main outcomes	Ref.
Bumah *et al.* (2015)†	MRSA	– 470 nm, 220 J/cm^2	– Total suppression for 220 J/cm^2 for both concentrations	[21]
Bumah *et al.* (2015)†	MRSA	– 405/470 nm, 60 J/cm^2	– 100% suppression of MRSA colonies achieved with 405 and 470 nm light	[22]
Chebath-Taub *et al.* (2014)	*Streptococcus mutans*	– 400–500 nm, 680 J/cm^2	– Delayed antibacterial influence of blue light; no effect upon capability of reforming new biofilm	[23]
Cieplik *et al.* (2014)†	*Aggregatibacter actinomycetemcomitans (Aa), Escherichia coli*	– 460 nm, 120 J/cm^2	– Inactivation >5 $\log_{10}$ for *Aa* (120 J/cm^2); different flavins and prophyrins in *Aa*	[11]
Cohen-Benneron *et al.* (2016)	*S. mutans*	– 460–480 nm, 262 J/cm^2	– Regrown biofilms after illumination less acidogenicity as well as lower aciduricity	[24]
de Sousa *et al.* (2015)	*E. coli, Pseudomonas aeruginosa, Staphylococcus aureus*	– 450 nm, 24 J/cm^2	– Inhibition of *S. aureus* (0.5 $\log_{10}$ for 6 J/cm^2) higher compared with *P. aeruginosa* and *E. coli*.	[25]
de Sousa *et al.* (2015)	*S. mutans*	– 420 nm, 72 J/cm^2	– Reduction of insoluble EPS highly affected by twice-daily blue light irradiation	[26]
de Sousa *et al.* (2016)	*S. aureus, E. coli, P. aeruginosa*	– 660/830/904 nm, 24 J/cm^2	– 24 J/cm^2 (660-nm red light): inhibition rate nearly 80% for *S. aureus*	[27]
Enwemeka *et al.* (2008)	MRSA	– 405 nm, 60 J/cm^2	– 55 J/cm^2: nearly 90% eradication	[28]
Enwemeka *et al.* (2009)	MRSA (two strains)	– 470 nm, 60 J/cm^2	– 55 J/cm^2 killed more than 90% of both strains	[29]
Feuerstein *et al.* (2005)	*Porphyromonas gingivalis, Fusobacterium nucleatum*	– 400–500 nm, 94 J/cm^2	– Significant reduction under aerobic conditions: nearly complete killing for illumination for 2.5 min (*P. gingivalis*)	[30]
Feuerstein *et al.* (2004)	*P. gingivalis, F. nucleatum, S. faecalis, S. mutans*	– 400–500 nm, 206 J/cm^2	– Higher inactivation rates for *P. gingivalis* compared with *F. nucleatum* (99.6% for 1 min with plasma arc)	[31]
Fontana *et al.* (2015)	*Fusobacterium/ Prevotella spp.*	– 455 nm, 4.8 J/cm^2	– Higher amounts of endogenous porphyrins for *Prevotella* species compared with *Fusobacterium* species	[32]
Fukui *et al.* (2008)	*P. gingivalis*	– 400–700 nm, 15 J/cm^2	– Irradiation using 400–410-nm light for 38 s at 400 mW/cm^2 more than 75% killing rate	[33]
Ghate *et al.* (2013)	*S. aureus, E. coli, S. typhimurium, L. monocytogens*	– 461/521/624 nm, 686 J/cm^2	– Approximately 5 $\log_{10}$ inactivation using 461-nm light at 10 and 15°C	[34]
Gomez *et al.* (2016)	*S. mutans*	– 405 nm, 9.26 J/cm^2	– Addition of sucrose leads to more resistant biofilms	[35]
Guffey and Wilborn (2006)	*S. aureus, P. aeruginosa, Propionibacterium acnes*	– 405/470 nm, 15 J/cm^2	– 405-nm light killed *S. aureus* at all the tested doses, no bactericidal effect with anaerobic *P. acnes*	[36]
Henry *et al.* (1995)	*Prevotella and Porphyromonas*	– 488–514 nm, 200 J/cm^2	– Black-pigmented bacteria (*Prevotella* and *Porphyromonas* species) most susceptible to visible-light irradiation	[37]

For further details, see Supplementary Table 2.
†In these studies, a reduction of 3 $\log_{10}$ steps or more (antibacterial effect [53]) was achieved. Details are presented in Table 2.
EPS: Extracellular polysaccharide; LED: Light-emitting diode; MRSA: Methicillin-resistant *Staphylococcus aureus*.

Table 1. Summary of all 34 selected studies sorted by authors (cont.).

Study (year)	Microorganisms	Wavelength, maximum energy	Main outcomes	Ref.
Henry *et al.* (1996)	Different bacterial species, *Candida*	– 488–514 nm, 35–80 J/cm^2	– Biofilm age, presence of oxygen, inoculation medium crucial for level of inactivation	[38]
Hope *et al.* (2013)	*P. gingivalis, E. faecalis*	– 405 nm, 98.55 J/cm^2	– Laser pointer: 94.50% killing for 98.55 J/cm^2	[39]
Hope *et al.* (2016)†	*P. intermedia, P. nigrescens, S. aureus, E. coli, E. faecalis*	– 405 nm, 5.7 J/cm^2	– *P. intermedia* 99.56% killed after 5 s, 99.996% after 60 s	[40]
Imamura *et al.* (2014)	*P. intermedia, P. gingivalis, E. coli, C. albicans*	– 405 nm, 6 J	– Inhibition rates for *P. intermedia* and *P. gingivalis* similar (maximum: 80%)	[41]
Izzo and Walsh (2004)	*P. gingivalis*	– 455/625 nm, 1.5 kJ/cm^2	– Temperature increase may be responsible for suppression of *P. gingivalis*	[42]
Kim *et al.* (2013)	*P. gingivalis, S. aureus, E. coli*	– 425/525/625 nm, 172.8 J	– 425 nm strongest effect followed by 525 nm; 625 nm no effect	[43]
König *et al.* (2000)	*Actinomyces odontolyticus, P. acnes, P. gingivalis, S. mutans*	– 632.8 nm, 360 J/cm^2	– Highest killing rate for *P. gingivalis* (50%)	[44]
Kotoku *et al.* (2009)†	*P. gingivalis*	– 405 nm, 16.0 J/cm^2	– 16.0 J/cm^2: complete eradication of *P. gingivalis*	[45]
Lipovsky *et al.* (2009)	*S. aureus* strains	– 400–800 nm, 180 J/cm^2	– Maximum inactivation rate: 99.8% at 180 J/cm^2	[46]
McKenzie *et al.* (2013)	*S. aureus, L. monocytogenes, P. aeruginosa*	– 405 nm, 504 J/cm^2	– Most rapid and effective inactivation for *E. coli* monolayer biofilms (3.55 $\log_{10}$ reduction for 20 min)	[47]
Maclean *et al.* (2008)†	*S. aueus, E. coli*	– >400 nm, 630 J/cm^2	– Maximum reduction at 405 nm at a total dose of 23.5 J/cm^2 (2.4 $\log_{10}$ steps)	[48]
Maclean *et al.* (2009)†	*S. aureus, S. pyogenes*, control organisms	– 405 nm, 54 J	– 5 $\log_{10}$ steps for *S. pyogenes* for 54 J/cm^2	[49]
Masson-Meyers *et al.* (2015)	MRSA	– 405 nm, 121 J/cm^2	– 405-nm light antimicrobial against MRSA, regardless if using LED or laser light	[50]
Song *et al.* (2013)†	*Aa, F. nucleatum, P. gingivalis*	– 400–520 nm, 6 J/cm^2	– Nearly 100% killing for 15 s in the case of *P. gingivalis*	[51]
Soukos *et al.* (2005)†	*Prevotall/ Porphyromonas spp., S. constellatus*	– 380–520 nm, 42 J/cm^2	–4.2 J/cm^2: complete killing of *P. intermedia* and *P. nigrescens* different endogenous porphyrins for *Prevotella* strains	[52]

For further details, see Supplementary Table 2.
†In these studies, a reduction of 3 $\log_{10}$ steps or more (antibacterial effect [53]) was achieved. Details are presented in Table 2.
EPS: Extracellular polysaccharide; LED: Light-emitting diode; MRSA: Methicillin-resistant *Staphylococcus aureus*.

light source in the first study [11], halogen lamp in the second study [51]) or due to different strains (ATCC 43718 [11] and ATCC 33384 [51], respectively) used for experiments.

As the first study clearly showed presence of flavins and porphyrins, killing by means of visible light could be a proper treatment modality for controlling infections caused by *A. actinomycetemcomitans*. In order to maintain the best results possible, light units showing broad overlap with the absorption spectra of both flavins and porphyrins should be used for irradiation.

Fusobacterium spp.

Song *et al.* tested *F. nucleatum* in planktonic state as well as in biofilm state [51]. Each bacterial sample was exposed to light from a halogen lamp (λ = 400–520 nm; output power of 500 mW/cm^2) for 15–120 s (7.5–60 J/cm^2). 99.1% reduction for planktonic bacteria was achieved after illumination for 60 s (<3 $\log_{10}$,

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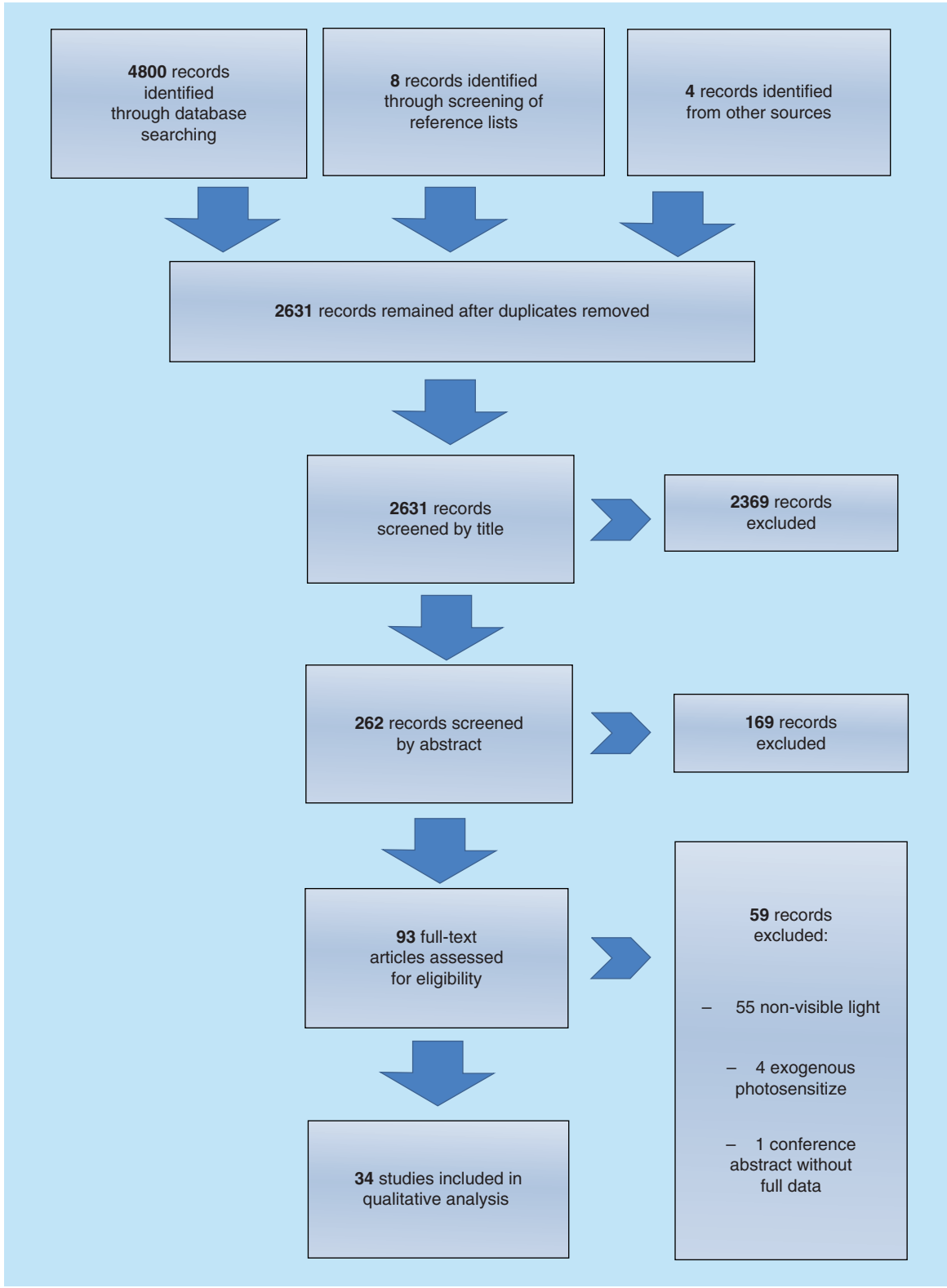

Figure 1. Flowchart of the search strategy as well as study selection and data management procedure.

no antibacterial effect [53]), whereas there was no effect on *F. nucleatum* organized as biofilm for any irradiation period tested. Inactivation rates were slightly higher for *P. gingivalis* under the same conditions (antibacterial effect [53]), whereas *A. actinomycetemcomitans* was not susceptible to visible light irradiation in any case (Table 1).

In another study, the effect of blue light from three different light sources was investigated for inactivation of planktonic *F. nucleatum* – two halogen lamps combined with filters (400–500 nm), a filtered xenon light source (plasma arc; 450–490 nm) and an LED (450–480 nm) [31]. Samples of bacteria in suspension and single bacteria on agar were exposed to light from the halogen lamps (260 and 416 mW/cm^2; 16–75 J/cm^2), the LED (520 mW/cm^2; 31–94 J/cm^2) and the plasma arc (1144 mW/cm^2; 69–206 J/cm^2). Corresponding irradiation periods for every sample were 1–3 min. As a result, higher inactivation rates could be detected when irradiation on agar plates was done in comparison to irradiation of planktonic cultures: 2.5 min with halogen lamp 2 resulted 'in nearly zero survival' [31] when irradiated on agar (no antibacterial effect [53]), whereas the survival rate was determined to be 40% when performed in suspension. Irradiation of *P. gingivalis* yielded higher inactivation rates compared with *F. nucleatum* (99.6% for 1 min with the plasma arc). Inactivation rates were below 99.9% in all cases meaning that no antibacterial effect was achieved [53].

In addition, in this study the effect of blue light was evaluated on a bacterial lawn (minimal fluence required for inhibiting bacterial lawn from growing into biofilm: minimal inhibitory dose [MID]). The MID for *P. gingivalis* and *F. nucleatum* was determined as 16–62 J/cm^2, whereas MID for *S. mutans* and *S. faecalis* was 159–212 J/cm^2 (~3 min), indicating that the *Streptococcus* strains tested were less susceptible to blue-light irradiation.

In a another study, the same group tested blue-light inactivation of bacteria under aerobic and anaerobic conditions as well as in presence of scavengers of ROS (dimethylthiourea, superoxide dismutase, ascorbic acid or a 'cocktail' of all three scavengers) on planktonic bacteria (for light sources, see [31]) [30]. There were no significant reduction rates for illumination under anaerobic conditions, whereas significant reduction could be detected in aerobic environment (nearly complete eradication for illumination for 2.5 and 3 min with LED for *P. gingivalis* and *F. nucleatum*; 3 min corresponding to 94 J/cm^2; no exact data presented, only figures). Effect for *F. nucleatum* was smaller compared with *P. gingivalis* for all experiments, suggesting that *P. gingivalis* might be more susceptible to killing by blue light (no antibacterial effect in any case [53]).

Addition of the scavengers only led to virtually smaller inactivation. For these experiments, only the halogen lamps and the plasma arc were used. The authors assumed that protection was incomplete due to inefficient access of the scavengers into the bacterial cells and inactivation might be due to photodynamic processes. In addition, poor inactivation under anaerobic conditions indicate that oxygen is necessary for inactivation of bacteria or that production of endogenous light-sensitive molecules may be downregulated under anaerobic conditions.

Table 2. Subset of nine studies, where an inactivation of ≥3 $\log_{10}$ steps† was achieved, sorted by the author.

Study (year)	Microorganism	Wavelength	Required dose for 3 $\log_{10}$-step reduction or more	Ref.
Bumah *et al.* (2015)	MRSA	470 nm	220 J/cm^2	[21]
Bumah *et al.* (2015)	MRSA	470 nm	55 J/cm^2	[22]
Cieplik *et al.* (2014)	*A. actinomycetemcomitans*	460 nm	150 J/cm^2	[11]
Ghate *et al.* (2013)	*S. aureus*	461 nm	597 J/cm^2	[34]
Hope *et al.* (2016)	*P. intermedia*	405 nm	20.6 J/cm^2	[40]
Kotokou *et al.* (2009)	*P. gingivalis*	405 nm	16 J/cm^2	[45]
Maclean *et al.* (2008)	*S. aureus*	>400 nm	420 J/cm^2	[48]
Maclean *et al.* (2009)	MRSA	405 nm	45 J/cm^2	[49]
	S. aureus	405 nm	36 J/cm^2	
	S. pyogenes	405 nm	54 J/cm^2	
Song *et al.* (2013)	*P. gingivalis*	400–520 nm	0.75 J/cm^2	[51]
Soukos *et al.* (2005)	*P. intermedia*	380–520 nm	4.2 J/cm^2	[52]
	P. melanogenica	380–520 nm	21 J/cm^2	
	P. nigrescens	380–520 nm	4.2 J/cm^2	

†Antibacterial effect [53].
MRSA: Methicillin-resistant *Staphylococcus aureus*.

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Fontana *et al.* used different *Fusobacterium* spp. for their experiments (*F. nucleatum ss. nucleatum*, *F. nucleatum ss. polymorphum*, *F. nucleatum ss. vincentii, F. periodonticum)* [32]. Irradiation of bacteria was performed using LED light at a wavelength of 455 nm at 80 mW/cm^2. Irradiation time was 60 s corresponding to an energy fluence of 4.8 J/cm^2. Killing rates were 46.9% for *ss. nucleatum*, 66.6% for *ss. peridonticum*, 67.4% for *ss. vincentii* and 93.6% *ss. polymorphium*, respectively. No antibacterial effect (3 $\log_{10}$-steps reduction or more [53]) could be shown for any of the tested species.

These studies suggest that different *Fusobacterium* strains might be susceptible to blue-light irradiation, but inactivation rates are lower compared with more pigmented genera (*Porphyromonas, Prevotella*). This might be due to lower amounts of endogenous porphyrins, as it was shown by the group of Fontana *et al.* [32].

Porphyromonas spp.

Kim *et al.* evaluated the efficacy of three different wavelengths for inactivating *P. gingivalis* (three-in-one mounted LED package, peaks at 425, 525 and 625 nm corresponding to blue, green and red light, respectively) [43]. Irradiation was performed for 0–24 h with an average output power of 6 mW/cm^2 per hour (48–144 J/cm^2). At a wavelengths of 425 and 525 nm, viability was decreased by 40–60% and 10–20%, respectively. No bactericidal effect was observed for 625 nm. 'For the suspensions containing 2 × 10^8 CFU/ml, the optical density $(OD)_{600}$ was decreased at 425 nm by 90–100% at all time points' [43] and 40–70% by 525-nm light after 8 h, whereas viability increased when irradiated with 625-nm light for 8 h. These results suggest that 425 nm is most effective in inactivating and suppressing *P. gingivalis*, followed by 525-nm light. 625-nm light did not show any effect for any case tested. As there is no accurate description concerning exact percentage of reduction, the observed effect cannot be declared as an antibacterial effect [53].

Feuerstein *et al.* investigated the phototoxicity of light from three different light sources (halogen lamp with a mounted filter, λ = 400–500 nm, 260 mW/cm^2; a filtered xenon light source, λ = 450–490 nm, 1144 mW/cm^2; LED, λ = 450–480 nm, 520 mW/cm^2) [30]. Using the LED, samples of *F. nucleatum* and *P. gingivalis* were irradiated for 1–3 min, corresponding to fluences of 31–94 J/cm^2, respectively. Inactivation only occurred under aerobic conditions (*P. gingivalis* nearly 80% for 1 min; for further details, see Table 1 and the *Fusobacterium* spp. chapter; no antibacterial effect [53] in any case).

In another study [31], the same group performed irradiation experiments using two halogen lamps combined with filters (Halogen 1 and Halogen 2; 260 and 416 mW/cm^2; λ = 400–500 nm), an LED and a filtered plasma arc (xenon light source; for details, see [30]). Irradiation periods were 1, 1.5, 2, 2.5 or 3 min, resulting in fluences of 16–75 J/cm^2 for the halogen lamps, 31–94 J/cm^2 for the LED and 69–206 J/cm^2 for the plasma arc. As a result, inactivation was higher with the LED and plasma-arc light source compared with the halogen lamp, which probably was due to the higher output powers (1144 and 520 mW/cm^2 for plasma arc and LED, respectively; 260 and 416 mW/cm^2 for halogen lamps; 99.6% killing efficacy in suspension for 1 min with the plasma arc for *P. gingivalis*; no antibacterial effect according to [53]; compare Table 1).

Henry *et al.* performed their experiments using an argon laser (λ = 488–514 nm; 20–200 J/cm^2) [37,38]. They were able to inactivate different *Prevotella* spp. (*P. intermedia* ATCC 15033 and 49046; *P. denticola* ATCC 33184) and *P. gingivalis* (strains ATCC 33277 and ATCC 350406) [37]. *P. intermedia* ATCC 15033 was most sensitive to irradiation 'with no cells surviving fluences of 70 J/cm^2 or greater' [37]. Because no exact data are presented, it was not possible to decide if there was an antibacterial effect [53]. In addition, they tested if replacement of hemin in the medium against hemoglobin had any influence in susceptibility to laser irradiation. They could show that one *P. gingivalis* strain (ATCC 33277) became tolerant to light irradiation in this case, indicating that hemin could be an essential factor for production of endogenous light-sensitive molecules. Also, the authors concluded that environmental oxygen is required for visible light inactivation. In general, nonblack-pigmented bacteria were much less sensitive to irradiation compared with black-pigmented bacteria

In a second study, the same group tested the susceptibility of bacteria organized in a biofilm grown on agar medium using the same argon laser [38]. Fluences of 35–80 J/cm^2 were able to inhibit biofilm growth of *P. endodontalis, P. gingivalis* as well as of different *Prevotella spp*, while there was no effect on the genera *Bacillus, Candida, Enterobacter, Proteus, Psuedomonas, Staphylococcus* and *Streptococcus* applying a fluence of 70 J/cm^2 (Table 1). The level of inactivation was affected by different parameters such as the biofilm age, presence of atmospheric oxygen or medium used for bacterial inoculation. Overall, the results were in accordance with the first study, suggesting that inactivation of different *Prevotella* and *Porphyromonas* species might be possible in planktonic as well as in biofilm state using an argon laser (no antibacterial effect [53] in any case).

Hope *et al.* irradiated a *P. gingivalis* suspension in 96-well plates [39]. Irradiation was performed using the LEDs from a 'toothcare device' (λ = 405 nm; power output: 3.2 mW) as well as a laser pointer (λ = 405 nm; power output: 42.7 mW) for 30, 60 and 300 s, corresponding to fluences of 0.34, 0.68 and 3.42 J/cm^2, respectively, for the toothcare device, whereas in the case of the laser 0.5, 1 and 5 min, corresponding to 9.86, 19.71 and 98.55 J/cm^2, respectively, were chosen. Irradiation with the LEDs was performed using *E. faecalis* as control organism under the same conditions as described for *P. gingivalis*. Irradiation at a fluence of 0.34 J/cm^2 with the LEDs resulted in a killing efficacy of 63.41%, whereas the killing rate at a fluence of 3.42 J/cm^2 was 94.11%. Using the laser pointer, killing rates were 90.21% for 9.86 J/cm^2 and 94.50% for 98.55 J/cm^2. There was no effect on *E. faecalis* upon irradiation. In neither case, there was an inactivation rate of 3 $\log_{10}$ steps or more [53].

Song *et al.* used a halogen lamp (dental-curing unit; 3M Curing Light XL3000, 3M ESPE, MN, USA; λ = 400–520 nm; 500 mW/cm^2) for irradiation of bacteria both in planktonic as well as in biofilm state (6.5 J/cm^2 up to 60 J/cm^2) [51]. The killing rate for *P. gingivalis* in planktonic state was below detection limit with an irradiation time of only 15 s (antibacterial effect [53], Table 2). When investigating visible light susceptibility of bacteria organized in a biofilm, only *P. gingivalis* could be killed using the same irradiation procedure as it was used with planktonic bacteria. *A. actinomycetemcomitans* and *F. nucleatum* were not susceptible to inactivation by visible light irradiation when organized as biofilms (no antibacterial effect [53] in any case).

Soukos *et al.* used a halogen lamp (λ = 380–520 nm; 70 mW/cm^2; 0–42 J/cm^2) for irradiation of different black-pigmented bacteria [52]. Survival fractions for *P. gingivalis* were 77.25% (4.2 J/cm^2), 12.55% (21 J/cm^2) and 1.48% (42 J/cm^2). Killing rates for the tested *Prevotella* species (*P. intermedia, P. nigrescens, P. elanogenica*) were higher compared with those of *P. gingivalis*. In contrast, *S. constellatus*, a nonpigmented species, was not killed under the same conditions, suggesting that pigmentation of bacteria is an important factor for visible light phototoxicity. No antibacterial effect [53] occurred in any case.

Fukui *et al.* aimed to determine the most effective wavelength for bacterial growth inhibition [33]. In course of this, planktonic *P. gingivalis* was exposed to monochromatic light using an Okazaki large spectrograph (λ = 400–700 nm in 10–20-nm steps; 50–400 mW/cm^2; 18 J/cm^2). Significant inhibition of *P. gingivalis* occurred between 400 and 410 nm, whereas no significant growth inhibition could be found when irradiation was performed at wavelengths longer than 500 nm. For example, irradiation using 400–410-nm light for 38 s at 400 mW/cm^2 resulted in a killing rate of more than 75% compared with nonirradiated control (no antibacterial effect [53]).

Fontana *et al.* exposed *P. gingivalis* to an LED emitting light at 455 nm (80 mW/cm^2) [32]. Irradiation time was 60 s corresponding to an energy fluence of 4.8 J/cm^2. The killing rate for *P. gingivalis* was 20.3% (no antibacterial effect [53]).

Furthermore, Kotoku *et al.* also performed phototoxicity experiments on *P. gingivalis* using a violet laser diode module of oscillating wavelength at 405 nm (200–800 mW/cm^2; 2.0–16.0 J/cm^2) [45]. An energy density of 4 J/cm^2 led to growth inhibition of more than 97%, while 16 J/cm^2 (20 s of irradiation) resulted in nearly complete eradication of *P. gingivalis* (antibacterial effect [53], Table 2). In general, higher output powers resulted in higher inactivation rates (400 mW for 5 s resulted in significant higher inactivation rates than 200 mW for 10 s; 2 J/cm^2, respectively).

Another study examined bacteria in suspension exposed to light from a helium–neon laser (λ = 632.8 nm red light; light intensity of 100 mW/cm^2; fluence of 360 J/cm^2) [44]. The killing rate for *P. gingivalis* was 41 ± 10% (70% ± 4% for *A. odontolyticus*; 42 ± 9% for *P. acnes*; no effect for *S. mutans*). Additional testing of susceptibility of wild-type *P. gingivalis* isolated from plaque samples showed a killing rate of 41 ± 26% (*Prevotella* spp. 58 ± 14%; *A. actinomycetemcomitans* 35 ± 17%). These results suggest that it is also possible to kill bacteria using red light, although the fluences used in the study were much higher than those used in most of the studies using blue light. In all cases, there was no antibacterial effect [53].

In contrast to the studies mentioned before, Izzo *et al.* assumed that killing of *P. gingivalis* was due to increasing temperatures, not due to a phototoxic effect. In this study, *P. gingivalis* was grown in a broth containing hemin and vitamin K [42]. For irradiation experiments, two different LEDs with peaks at wavelengths of 455 ± 20 or 625 ± 20 nm were used. Tubes containing bacteria were irradiated with a total dose of 978 J/cm^3 (625 ± 20 nm) or 1.5 kJ/cm^3 (455 ± 20 nm) in a temperature-controlled shaker. Temperature during irradiation was 39–40°C for blue light and 41–42.5°C for red light. Consequently, the authors suggested that the increase of temperature was responsible for suppression of *P. gingivalis* and not a phototoxic effect due to excitation of endogenous PS. However, it has to be considered that these light doses were very high which may explain the observed temperature increases.

Overall, these results show that *P. gingivalis* was susceptible to blue- and red-light irradiation in most of the studies, although an antibacterial effect [53] was

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observed in only two studies [45,51] (Tables 1 & 22). Inactivation rates for blue light were higher in general, but red-light irradiation could be particularly useful as it is known that light of longer wavelengths is able to penetrate tissues to a deeper extent [55].

Prevotella spp.

Imamura *et al.* investigated the effect of blue light on *P. intermedia* [41]. Irradiation was performed using a 405-nm monochromatic laser (300 s; 0.05–60 J). Inhibition rates were 40% for 15 J, ~70% for 30 J and ~80% for 45 and 60 J (no antibacterial effect [53]). Inhibition of *P. intermedia* was similar to the results found for *P. gingivalis*, while there was no effect for suspensions of *E. faecalis*. There was no antibacterial effect [53] in any case.

Another group used planktonic *P. intermedia, P. elanogenica* and *P. nigrescens* grown anaerobically for their experiments (halogen lamp; 70 mW/cm^2; 0–42 J/cm^2) [52]. Irradiation for 1 min (4.2 J/cm^2) resulted in an inactivation below detection limit for *P. intermedia* and *P. nigrescens*, while *P. melanogenica* was reduced by 70%. Inactivation below detection limit of the latter was achieved by 5 min of irradiation (21 J/cm^2; antibacterial effect [53], Table 2). Survival fractions for *P. gingivalis* were 77.25% (4.2 J/cm^2), 12.55% (21 J/cm^2) and 1.48% (42 J/cm^2), while there was no effect in the case of *S. constellatus*. HPLC analysis showed endogenous porphyrins in *P. intermedia*, *P. nigrescens* and *P. melanogenica* [52].

Hope *et al.* tested strains of *P. intermedia* and *P. nigrescens* [40]. Irradiation was performed using an LED (19.1 mW/cm^2, λ = 405 nm) and a laser pointer (346.2 mW/cm^2, λ = 405 nm). Irradiation periods were 5–60 s for the laser (1.7–20.8 J/cm^2) and 10–300 s for the LED (0.19–5.7 J/cm^2). Control organisms (*E. coli, Staphylococcus aureus, E. faecalis*) were irradiated for 300 s using the LED (5.7 J/cm^2). Killing rates for *P. nigrescens* for the LED were 64.1% after 30 s and 94.26% after 300 s. In the case of *P. intermedia*, even higher killing rates were observed using the LED (75.62% after 10 s, 96.51% after 60 s, 99.75% after 300 s). Using the laser pointer, 99.56% of bacteria were killed after 5 s and 99.996% after 60 s (antibacterial effect [Lit], Table 2). Higher inactivation rates using the laser might be due to the much higher output power compared with the LED (346.2 mW/cm^2 as compared with 19.1 mW/cm^2). With respect to the control organisms, only in the case of *S. aureus*, a statistically significant antimicrobial effect could be observed (36.73% after 300 s, no antibacterial effect [53]).

Fontana *et al.* used different *Prevotella* spp. for their experiments (*P. intermedia, P. melanogenica, P. nigrescens*; LED; λ = 455 nm; 80 mW/cm^2; 60 s; 4.8 J/cm^2) [32]. Survival fractions were 46.2% for *P. ntermedia*, 32.5% for *P. nigrescens* and 21.3% for *P. melanogenica*, respectively. The lowest survival rate was shown for *F. nucleatum ss. polymorphum* (6.4%), while the highest survival rate was shown for *P. gingivalis* (79.7%; no antibacterial effect [53] in any case). Additional examination of endogenous porphyrin production showed 80- to 200-times higher amounts for *Prevotella* spp. compared with *Fusobacterium* spp.

Henry *et al.* performed experiments using an argon laser (λ = 488–514 nm; 20–200 J/cm^2) for inactivation experiments of different *Prevotella* and *Porphyromonas* strains (please see Table 1 and *Porphyromonas* spp. chapter) [37,38].

König *et al.* evaluated the effect of red light on wild-type germs, isolated from patients with periodontal disease (helium–neon laser, λ = 632.8 nm, 360 J/cm^2) [44]. The killing rate for *Prevotella* spp. was determined to be 58 ± 14% (no antibacterial effect [53]).

Overall, these results show that *Prevotella* spp. might be susceptible to irradiation with blue and red light, although no antibacterial effect [53] could be found in any of the studies. Visible light could be an adjunctive method in the treatment of diseases associated with the presence of these germs. As it was shown that inactivation might be possible using blue light and also red light, a combination of blue and red light could be useful for inactivation of *Prevotella* species as this combination would combine maximum bactericidal effect and maximum tissue penetration, which is known to be higher for light of longer wavelengths [55].

Staphylococcus spp.

de Sousa *et al.* investigated the effect of blue light emitted from a laser (λ = 450 nm; 70 mW) on strains of *S. aureus* [25]. Growth was inhibited at fluences higher than 6 J/cm^2 (reduction of 22% for 6 J/cm^2; no higher inhibition rates detected for 12, 18 and 24 J/cm^2; no antibacterial effect [53] in any case). In general, inhibition of *S. aureus* was higher than inhibition observed for the control organisms *Pseudomonas aeruginosa* and *E. coli*.

Maclean *et al.* observed a significant bactericidal effect with a 5 $\log_{10}$-step reduction (antibacterial effect [53]) upon illumination *of S. aureus* with a broadband xenon light source (30 min; 350 mW/cm^2, 630 J/cm^2; 3 $\log_{10}$ reduction for 420 J/cm^2) [48]. Further experiments showed no effects for wavelengths longer than 430 nm, suggesting that only a small fraction within the wavelength range emitted by the broadband light source was responsible for inactivation. Maximum reduction in dependence of applied wavelength was observed at 405 nm at a fluence of 23.5 J/cm^2 (2.4 $\log_{10}$ steps). Besides, they tested two

different MRSA strains (a laboratory strain and a clinical strain obtained from a wound infection) for susceptibility under the same conditions. Results were similar to those obtained with *S. aureus* [48] (no detailed data presented). According to the study, there was an antibacterial effect [53] with the broadband xenon light for 420 J/cm^2. For *E. coli*, which was used as reference strain, there was no inactivation at all for irradiation up to 30 min.

In another study by the same group, selected medical pathogens (please see Table 1) were illuminated with light from an arrow of LEDs (10 mW/cm^2) at a wavelength of 405 nm [49]. An approximately 5 $\log_{10}$-step reduction of CFU counts was achieved following exposure for 60–90 min (36–54 J/cm^2). As a result of all the tested bacteria, they concluded that lower doses are sufficient for inactivation of Gram-positive bacteria, with the exception of *E. faecalis*, which was least susceptible to irradiation performed at 405 nm. They concluded, that light emitted from an arrow of LEDs at 405 nm may be able to completely eradicate bacteria at high-population densities. For methicillin-suceptible *S. aureus*, 36 J/cm^2 was needed for a 5 $\log_{10}$-step reduction, whereas 45 J/cm^2 was necessary in order to reach the same effect for MRSA (antibacterial effect [53]).

Using violet and blue light (λ = 405 and 470 nm) from superluminescent diodes at different fluences (1–15 J/cm^2), Guffey *et al.* observed nearly 90% of bacteria killing for *S. aureus* strains at a fluence of 15 J/cm^2 at 405 nm (160 mW) [36]. 405-nm light killed *S. aureus* at all the tested fluences, whereas 470 nm (150 mW) showed an effect at 10 and 15 J/cm^2 only exhibiting a 62% killing rate at 15 J/cm^2. Irradiation of *P. aeruginosa* resulted in higher inactivation rates at all doses (95.1% maximum killing rate for 405 nm, 96.5% for 470 nm), suggesting that *P. aeruginosa* might be slightly more susceptible (no antibacterial effect [53]) to violet- and blue-light irradiation. In contrast, no effect was reported upon irradiation of *P. acnes*, which is in contrast to several other *in vitro* and *in vivo* studies [56,57].

Kim *et al.* performed experiments using 425, 525 and 625 nm light on *P. gingivalis*, *S. aureus* and *E. coli* [43]. Results suggest that 425 nm as well as 525 nm light might be able to inactivate bacteria with higher susceptibility for *P. gingivalis* and *E. coli* compared with *S. aureus* (for details, please see Table 1 and *Porphyromonas* spp. chapter).

In another study, irradiation with different wavelengths from the visible and near-infrared spectrum (660 nm red light, 830 and 904 nm near-infrared) emitted from a laser was tested for its effect in inhibiting growth of *S. aureus*. Inhibition of growth was observed for fluences higher than 12 J/cm^2 [27]. At a fluence of 24 J/cm^2 (660 nm red light; 30 mW output power), the inhibition rate was nearly 80% (no antibacterial effect [53]). Red light was more effective than infrared light. *S. aureus* was more susceptible to irradiation at all tested wavelength regardless of the applied fluence than *P. aeruginosa* and *E. coli* (no antibacterial [53] effect in any case).

Liposvky *et al.* tested two different strains of *S. ureus* for their susceptibility to visible light emitted from a halogen lamp (300 mW/cm^2; 1, 5, 10 min; 18–180 J/cm^2), one methicillin-sensitive and one methicillin-resistant strain [46]. Results showed that the methicillin-sensitive strain was more susceptible to white light irradiation than the methicillin-resistant strain, with a maximum inactivation rate of 99.8% at a fluence of 180 J/cm^2 compared with 55.5% (no antibacterial effect [53] in any case). Measurements of hydroxyl and superoxide radical production in illuminated bacteria as well as porphyrin synthesis showed higher amounts for the methicillin-sensitive strain, whereas the resistant strain was able to adapt to oxidative stress to a higher extent. Carotinoid production was also measured, exhibiting higher values for the resistant strain. These findings may explain the higher susceptibility of the methicillin-sensitive strain to visible light irradiation.

Emwemeka *et al.* used a 470-nm SLD (superluminescent diode) phototherapy device (30 mW/cm^2; 1–60 J/cm^2) for experiments with two strains of MRSA [29]. At a fluence of 3 J/cm^2, nearly 30% inactivation was found for both strains. 55 J/cm^2 led to more than 90% killing rate for both strains (no antibacterial effect [53] in any case).

In another study performed by the same group, two strains of MRSA were irradiated with violet light [28]. As a light source, a cluster of 36 SLDs emitting light ranging from 390 to 420 nm with a 405-nm peak was used (average power: 500 mW; irradiance: 100 mW/cm^2; 1–60 J/cm^2). Maximum eradication was achieved applying light for 9.2 or 8.4 min with 55 J/cm^2 resulting in a killing rate of nearly 90% (no antibacterial effect [53] in any case).

The same group used MRSA spread on tryptic soy agar for irradiation experiments with a 470-nm blue light-emitting LED (30 mW/cm^2; 55–220 J/cm^2) [21]. 55 J/cm^2 resulted in 86–92% inactivation while 110 J/cm^2 and 220 J/cm^2 resulted in total suppression regardless of the cell concentration (antibacterial effect [53], Table 2).

Bumah *et al.* used an MRSA isolate for experiments with violet and blue light (405 and 470 nm) [22]. Bacteria were spread on agar plates before irradiation. 100% of MRSA colonies at a concentration of 3 × 10^6 cells/ml were suppressed by a single exposure to 55 or 60 J/cm^2

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of 470-nm light; double exposure to 405-nm light with a 6-h interval at 50, 55 or 60 J/cm^2 showed the same result. Furthermore, this double treatment resulted in suppression of a cell density of 5 × 10^6 CFU/ml, which was below detection limit (antibacterial effect [53]). 7 × 10^6 CFU/ml density in contrast had to be illuminated either once with 220 J/cm^2 of 470-nm blue light or twice with 220 J/cm^2 using 405-nm blue light in order to achieve the same effect. The authors concluded that repeated illumination may be necessary for complete eradication of denser bacterial concentrations, especially when applying lower fluences (Tables 1 & 2).

Masson-Meyers *et al.* compared the effect of 405-nm blue light emitted from an LED (100 mW/cm^2) with blue light emitted from a laser (135 mW/cm^2) on MRSA (5 × 10^6 cells/ml) [50]. Irradiation was performed once, twice or thrice with either light from the LED or from the laser at fluences of 40, 54, 81 or 121 J/cm^2. Time intervals in between were 15, 30 or 240 min. Results showed significant growth suppression for each fluence for both light sources with no 'statistical difference' for LED and laser 'in 35 of the 36 experimental samples' [50]. Irradiation in two or three intervals increased bacterial suppression, especially when the treatment interval was 15 or 30 min. 54 J/cm^2 triple irradiation with laser (intervals of 15 min) resulted in suppression, which was below detection limit (antibacterial effect [53], Table 2).

Testing susceptibility of monolayer biofilms formed by different bacteria on glass surfaces, McKenzie *et al.* performed irradiation for 5, 10 and 20 min using a 405-nm LED (141.48 mW/cm^2) [47]. Inactivation rates for *S. aureus* were 0.61, 1.87, 2.75 $\log_{10}$ steps for 5, 10 and 20 min (0.7, 1.4, 2.8 J/cm^2), respectively (no antibacterial effect [53]). Most rapid and effective inactivation was observed for *E. coli* monolayer biofilms with a 2.52 $\log_{10}$-step reduction upon 10 min of exposure to light, and 3.55 $\log_{10}$-step reduction following 20 min of exposure (antibacterial effect [53]). In the case of mixed-species biofilms formed by strains of *S. aureus* and *E. coli*, a 2.19 $\log_{10}$-step reduction (total viable counts) after 30 min of irradiation was achieved.

Ghate *et al.* tested the antibacterial effect of LEDs emitting light in the visible region (7.5 h; 461 nm, 16 mW/cm^2, 432 J/cm^2; 521 nm, 22,1 mW/cm^2, 557 J/cm^2; 642 nm, 25,4 mW/cm^2, 640 J/cm^2) on *S. aureus* as well as selected foodborne pathogens (*E. coli*, *S. typhimurium*, *L. monocytogens*) [34]. Irradiance was performed at three different temperatures (20, 15 and 10°C). An approximately 5 $\log_{10}$-step inactivation (antibacterial effect [53]) for all the tested strains was observed using 461-nm light at 10 and 15°C, while inactivation rates for 521 nm light were 1–2 $\log_{10}$ steps only and there was no antibacterial effect [53] for 642-nm light. In general, inactivation rates were higher at 10 and 15°C compared with 20°C.

It can be concluded that *Staphylococcus* spp. may be susceptible to visible light irradiation to a certain extent. Especially in the case of strains resistant to common antibiotics (MRSA), this might be a useful additional treatment modality.

Streptococcus spp.

Maclean *et al.* used high-intensity 405-nm light from an LED (10 mW/cm^2) for irradiation of different Gram-positive and Gram-negative bacteria [49]. Highest inactivation rates were measured for *Streptococcus*, *Staphylococcus* and *Clostridium* spp. (~5 $\log_{10}$-step reduction following irradiations for 60 and 90 min corresponding to fluences of 36 and 54 J/cm^2; antibacterial effect [53]).

Soukos *et al.* used strains of *S. constellatus* for their experiments [52]. For illumination of planktonic cultures, a halogen lamp (λ = 380–520 nm, 70 mW/cm^2, 0–42 J/cm^2) was used. There was no effect for visible light irradiation for *S. constellatus*.

Feuerstein *et al.* compared susceptibility of planktonic cultures of *P. gingivalis*, *F. nucleatum*, *S. mutans* and *E. faecalis* to blue light emitted from halogen lamps with filters (400–500 nm; 260–416 mW/cm^2), a filtered xenon light source (plasma arc: 450–490 nm; 1144 mW/cm^2) and an LED (450–480 nm; 520 mW/cm^2) [31]. As a result, bactericidal effects for *P. gingivalis* and *F. nucleatum* could be observed only, whereas there was no effect for *S. mutans* and *S. faecalis* under the same conditions (Table 1; no antibacterial effect [53]).

Examination of viability and structure of biofilms of *S. mutans* formed after exposure to blue-light irradiation for 1–10 min (400–500 nm; 1.13 W/cm^2; 68–680 J/cm^2) was investigated by Chebath-Taub *et al.* [23]. Illuminated biofilms were dispersed and reorganization as a new biofilm was examined at different time intervals by viable counts and confocal laser scanning microscopy (LIVE/DEAD staining). As a result, a significant decrease in bacterial viability was found after 6 h of reorganization (up to 80% when irradiated for 10 min before reorganization, no antibacterial effect [53]). Besides, the amount of dead bacteria increased compared with the situation before irradiation, suggesting that blue light might have a delayed antibacterial influence although there was no effect upon capability of reforming new biofilm.

In a subsequent study, this group performed further tests concerning the pathogenicity of the new formed biofilms (LED light-curing unit; λ = 460–480 nm; 620 mW/cm^2; 1, 3, 7 min; 37, 112 and 262 J/cm^2) [24]. Quantification of bacteria was

achieved by measurement of the optical density and quantitative polymerase chain reaction (quantification of DNA samples of *S. mutans*); furthermore, confocal laser scanning microscopy was used for determination of bacterial viability and extracellular polysaccharide (EPS) production; quantitative polymerase chain reaction was also used for determination of acidogenicity and acidurity of bacteria. Although bacterial total growth increased in regrown biofilms, amount of dead bacteria outweighed while polysaccharide production decreased. Regrown biofilms after illumination showed less acidogenicity as well as lower aciduricity. According to these results, blue light may be a proper treatment tool for reducing pathogenicity of bacteria dispersed from the biofilm-colonizing new surfaces. It may not be possible to completely kill bacteria organized in a biofilm structure, but it may be possible to diminish the pathogenicity of biofilms by means of visible light irradiation.

In another study, Gomez *et al.* irradiated initial biofilms (λ = 380–440 nm with 405-nm peak) formed by strains of *S. mutans* grown for 12–16 h [35]. Intensity on surface was 13 mW/cm^2 and irradiation was done for 5 min, resulting in a fluence of 9.26 J/cm^2. Biofilms were grown either in Tryptic Soy Broth (TSB) or in TSB containing 1% sucrose. Percentage of bacteria killed was 70% in the case of biofilm grown in TSB and 50% in the case of biofilm grown in TSB containing 1% sucrose suggesting that addition of sucrose leads to more resistant biofilms (no antibacterial effect [53]). These results show that killing of bacteria not only depends on the microorganism by itself but also on other factors such as nutrition.

In another study, bacteria were exposed to red light emitted from a helium–neon laser (60 mW helium–neon laser; 632.8 nm; total energy density: 360 J/cm^2) [44]. While there were bactericidal effects on *A. odontolyticus, P. acnes* and *P. gingivalis*, there was no effect on *S. mutans* (no antibacterial effect [53] in any case).

Aim of another work was to examine twice-daily visible light irradiation on development of matrix-rich biofilms [26]. *S. mutans* biofilms were grown for 5 days. Irradiation was performed two-times a day using a noncoherent blue-light source (420-nm blue light; 92 mW/cm^2; 12 min 56 s; 72 J/cm^2). Bacterial viability, dry weight and intracellular polysaccharide (IPS) and EPS were measured. Twice-daily treatment with 0.12% chlorhexidine served as positive control, while NaCl treatment served as negative control (1 min, respectively). CFUs were reduced to the highest amount in the chlorhexidine group (around 4 $\log_{10}$ steps, antibacterial effect [53], Table 2), while there was only minimal reduction when irradiated with blue light (~1 $\log_{10}$ step; no antibacterial effect [53]). Reduction of insoluble EPS was highly affected by twice-daily blue-light irradiation, suggesting that this might be a proper treatment modality in prevention of biofilm development as insoluble EPS is forming the scaffold of the extracellular biofilm matrix.

Overall, the results obtained for visible light irradiation of *Streptococcus* spp. suggest that eradication, especially in biofilm state, might be difficult to achieve, but visible light could be a promising modality for influencing biofilm architecture in order to lower pathogenicity, aciduricity and acidogenicity.

Discussion & conclusion

Visible light irradiation is already regularly applied in clinical dermatological practice for topical treatment of acne vulgaris [56,57] or infected wounds [58]. Furthermore, visible light application is discussed as a treatment option for patients with gastric diseases mainly caused by *Helicobacter pylori*, where traditional treatment modalities often lead to failure [59,60]. The aim of this study was to summarize studies dealing with *in vitro* inactivation of bacteria occurring in the oral cavity in order to estimate the potential of visible light for inactivation of oral diseases associated with bacteria.

As a result, it can be concluded that eradication of bacteria in planktonic cultures by means of visible light seems possible, especially in case of black-pigmented bacteria such as *Porphyromonas* and *Prevotella* spp. With regard to bacteria organized in biofilms, reported evidence is less clear. Considering the reviewed studies, it seems as if inactivation is hampered when bacteria are embedded in a biofilm structure. This has to be taken into consideration as biofilm accumulation is the most common situation in the oral cavity as well as in nature [61]. It is well known that biofilms show higher resistances to distinct antibacterial agents [62]. In this instance, the biofilm matrix has been identified as a possible barrier for penetration of antibacterial agents [63]. If an antibacterial effect [53] is required, for example, for endodontic issues [64], additional application of an exogenous PS may result in higher inactivation rates [65,66]. Thereby, the antimicrobial effect of the light source itself should be seen as an additional effect resulting in higher inactivation rates. In this respect, light offers a synergistic effect to the PS. If light of distrinct wavelengths without PS is used for bacterial inactivation high levels of energy are required, which may result in potential tissue damage [67]. In light of this, a novel light source emitting visible light + water-filtered infrared-A is potentially beneficial, particularly in combination with a PS, for treatment of topical biofilm infections [68], as water-filtered infrared-A is also known for its wound healing properties [69].

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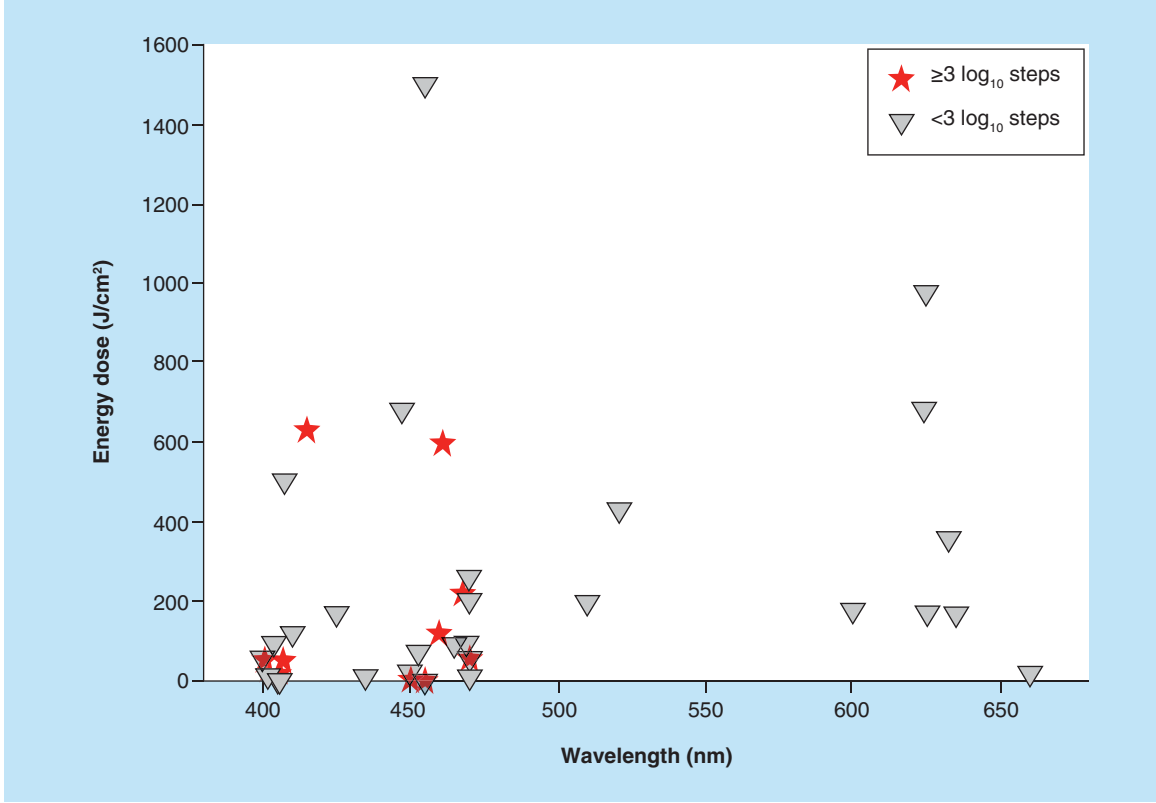

Figure 2. Wavelengths used for inactivation as well as corresponding maximum energy dose or energy dose required for an inactivation ≥3 log_{10} steps (asterisk) and <3 log_{10} steps (triangle) are shown (planktonic bacteria). For wavelength, peak showing maximum output power of the light source is shown whenever spectral characteristics were known, otherwise median wavelengths are shown. Further details are shown in Supplementary Table 1.

Considering effectiveness of light of different wavelengths, there seems to be an overlap of emission spectra with absorption spectra of different classes of porphyrins [70,71]. This is in accordance with different reviewed studies which showed high amounts of endogenous porphyrins for different bacteria [11,32–33].

In contrast, for prophylactic issues illumination with visible light without an exogenous PS may be a useful tool by controlling pathogenicity of biofilms [32]. The great advantage of visible light consists in the possibility of repetitive treatments as there are no major side effects as compared with UV light, for example [14]. Besides, application of light without preincubation with an exogenous PS is a treatment modality that may even be conducted by a patient by his or her own due to its ease in handling. For being able to draw a conclusion on the potential of this modality, further *in vitro* studies are necessary that investigate the influence of visible light irradiation on biofilms, for example, formed *in vitro* by cariogenic or periodontal key pathogens or on biofilms *ex vivo*.

Visible light could be a promising treatment option for periodontal disease, where black pigmented bacteria play a crucial role [72]. By selectively killing black-pigmented bacteria, it could be possible to lower pathogenicity of a periodontal biofilm without harming beneficial bacteria resulting in a healthy equilibrium [52].

It has to be considered, though, that the energy doses and irradiation times applied in some studies are beyond practicability, lowering the value of the received results. Using high-energy doses, it is sometimes not clear whether inactivation occurs due to light-mediated effects or due to heat-induced effects.

As there is a great variation in the energy doses applied in the studies included in this review, comparison of the obtained results is seriously hampered (compare Tables 1 & 2). Therefore, for further studies, it is a crucial point to take the characteristics of the employed light sources into account. Proper comparison of antibacterial properties of light of distinct wavelengths on microorganisms will only be possible, if specific characteristics of the light source, like wavelength, emission spectrum and fluence are taken into consideration. In this regard, it has recently been shown by our group in the case of photodynamic inactivation in presence of an exogenous PS that this should be considered for

comparing antibacterial activity with different PSs and light of different wavelengths [73]. It could be useful to establish a similar formula for comparison of phototoxic effect of distinct bacteria by illumination with light without addition of an exogenous PS.

Another point is that culture conditions of the bacteria investigated in the studies considered are highly variable, too. It is well known that production of endogenous porphyrins is highly depending of the respective culture conditions (e.g., addition of precursors in porphyrin-synthesis like hemin and vitamin K) [74].

Overall, the results reviewed in this paper imply that visible light could be a treatment option for oral diseases. However, further studies especially concerning the situation in biofilm state are necessary, before clinical studies can be conducted for a final assessment of effectiveness of visible light irradiation for treatment of oral diseases.

Future perspective

Application of visible light may be a promising tool for control of oral bacterial infections, especially considering prophylactic issues. As already concluded before, further studies especially dealing the situation in a biofilm state are required.

Considering the situation with addition of an exogenous light-sensitive substance, the toxicity of the light itself has to be seen as an additional improvement for *in vivo* use. Therefore, it seems appropriate for development of new light-sensitive substances to use light of a wavelength that shows antimicrobial effects in order to further enhance the effect.

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Executive summary

- Inactivation of bacteria in planktonic state shows promising results.
- There is less evidence considering situation in a biofilm; inactivation using visible light seems less effective when bacteria are organized as a biofilm.
- Inactivation may be due to endogenous porphyrins produced during bacterial metabolism.
- Use of visible light should be taken into consideration, especially for routine use in prophylactic issues.

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