

Methylene blue-mediated photodynamic therapy in the treatment of oral microbiota.

A Systematic Review

A terapia fotodinâmica mediada pelo azul de metileno no tratamento da microbiota bucal. Uma

Revisão Sistemática

Terapia fotodinámica mediada por azul de metileno en el tratamiento de la microbiota oral. Una

Revisión Sistemática

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Abstract

Objective: The objective of the present review was to relate the scientific production presented in the last 5 years on the effect of photodynamic therapy using the methylene blue photosensitizer on the microorganisms present in the oral biofilm. **Methods:** systematic review looking for research in English of primary studies such as randomized clinical trials and without randomization, individual case-control, and qualitative descriptive studies, using the PubMed/MEDLINE and Scopus databases as references, from 2017 to 2021. **Results:** 15 articles were analyzed, being 67% national publications and 33% international studies. Considering the samples described in the studies, 73% were in vitro, 7% were in vivo and 20% were clinical samples. As for the light source, 66% used laser, 20% used LED and 14% the association of laser and LED devices. The power density used ranged from 0.0026 W/cm² to 1415 W/cm². The energy density ranged from 3 J/cm² to 640 J/cm². The power ranged from 10 mW to 110 mW. The irradiation time ranged from 24 to 294 seconds. In most publications, 87% used the wavelength of 660 nm. As for the photosensitizer, the most used concentration, in 33% of the studies, was 0.01%, with the pre-irradiation time most used being 300 seconds in 40% of the studies. **Conclusion:** Photodynamic Methylene Blue Therapy is being accepted as effective in therapies of various oral curative conditions and has been shown to be a useful and effective therapy, as well as a complementary approach to control the development/growth of oral biofilm in clinical situations.

Keywords: Photochemotherapy; Photosensitizer; Mouth; Biofilms.

Resumo

Objetivo: O objetivo da presente revisão foi relacionar a produção científica apresentada nos últimos 5 anos sobre o efeito da terapia fotodinâmica utilizando o fotossensibilizador azul de metileno sobre os microrganismos presentes no biofilme oral. **Métodos:** Revisão sistemática buscando pesquisas em inglês de estudos primários como ensaios clínicos randomizados e sem randomização, caso-controle individual e estudos descritivos qualitativos, tendo como referências as bases de dados PubMed/MEDLINE e Scopus, no período de 2017 a 2021. **Resultados:** Foram

identificados 15 artigos, sendo 67% publicações nacionais e 33% internacionais. Considerando as amostras descritas nos estudos, 73% eram *in vitro*, 7% eram *in vivo* e 20% eram amostras clínicas. Quanto à fonte de luz, 66% utilizaram laser, 20% utilizaram LED e 14% a associação de laser e LED. A densidade de potência utilizada variou de 0,0026 W/cm² a 1415 W/cm². A densidade de energia variou de 3 J/cm² a 640 J/cm². A potência variou de 10 mW a 110 mW. O tempo de irradiação variou de 24 a 294 segundos. Na maioria das publicações, 87% empregaram-se o comprimento de ondas de 660 nm. Quanto ao fotossensibilizador, a concentração mais utilizada, em 33% dos estudos, foi de 0,01%, sendo o tempo pré-irradiação mais utilizado de 300 segundos em 40% dos estudos. Conclusão: A Terapia Fotodinâmica com azul de metileno está sendo aceita como eficaz em terapias de diversas condições curativas orais e tem se mostrado uma terapia útil e eficaz, bem como uma abordagem complementar para controlar o desenvolvimento/crescimento do biofilme oral em situações clínicas.

Palavras-chave: Fotoquimioterapia; Fotossensibilizador; Boca; Biofilmes.

Resumen

Objetivo: El objetivo de la presente revisión fue relacionar la producción científica presentada en los últimos 5 años sobre el efecto de la terapia fotodinámica utilizando el fotosensibilizador azul de metileno sobre los microorganismos presentes en el biofilm oral. Métodos: revisión sistemática buscando investigaciones en inglés de estudios primarios como ensayos clínicos aleatorizados y sin aleatorización, estudios descriptivos individuales de casos y controles, utilizando como referencias las bases de datos PubMed/MEDLINE y Scopus, de 2017 a 2021. Resultados: 15 artículos fueron analizados, siendo un 67% publicaciones nacionales y un 33% estudios internacionales. Considerando las muestras descritas en los estudios, el 73% fueron *in vitro*, el 7% fueron *in vivo* y el 20% fueron muestras clínicas. En cuanto a la fuente de luz, el 66% utilizó láser, el 20% LED y el 14% la asociación de dispositivos láser y LED. La densidad de potencia utilizada osciló entre 0,0026 W/cm² y 1415 W/cm². La densidad de energía osciló entre 3 J/cm² y 640 J/cm². La potencia osciló entre 10 mW y 110 mW. El tiempo de irradiación osciló entre 24 y 294 segundos. En la mayoría de las publicaciones, el 87% utilizó la longitud de onda de 660 nm. En cuanto al fotosensibilizante, la concentración más utilizada, en el 33% de los estudios, fue del 0,01%, siendo el tiempo de preirradiación más utilizado de 300 segundos en el 40% de los estudios. Conclusión: La terapia fotodinámica con azul de metileno está siendo aceptada como efectiva en terapias de varias condiciones curativas orales y ha demostrado ser una terapia útil y efectiva, así como un enfoque complementario para controlar el desarrollo/crecimiento de biopelícula oral en situaciones clínicas.

Palabras clave: Fotoquimioterapia; Fotosensibilizante; Boca; Biopelículas.

1. Introduction

Several oral alterations are interdependent diseases of microbial biofilms that play a vital role in the development of caries, periodontitis and other inflammatory processes, and can be defined as a well-structured ecosystem allowing microorganisms to thrive in adverse environmental conditions. Consequently, this microbiota, when associated with the biofilm, develops greater antibiotic resistance, influencing the success of treatments (Huang et al., 2019; Warriar et al., 2021;).

Colonized by bacterial, fungal and viral species, and found in practically every niche in the oral cavity, the biofilm has the dorsum of the tongue and the surfaces of the teeth as the main sites and can develop on any surface that is in the oral cavity such as dentures, orthodontic appliances and orogastric tubes. Formed by more than 400 microbial species, it has a high local and systemic pathogenic potential, providing protection to multidrug-resistant microorganisms, including against antibacterial agents (Lobão et al., 2016). The insoluble polysaccharide matrix is the primary factor that can hinder the diffusion and access of antimicrobial agents (Huang et al., 2019; Warriar et al., 2021).

The growing antimicrobial resistance due to the indiscriminate use of antibiotics led to several studies using Photodynamic Therapy (PDT-Photodynamic Therapy). In this therapy, a chemical product (dye) is used and excited by light, promoting cell apoptosis (Lacerda et al., 2014). Apoptosis causes programmed cell death with cell retraction due to the loss of adhesion with other cells and with the extracellular substance, and subsequent formation of extensions in the cell membrane that rupture with formation of vesicles (apoptotic bodies) that are later phagocytosed by macrophages. This process does not promote cell disruption or dissolution and does not cause leakage of cytoplasmic content, thus avoiding tissue damage, which makes PDT an effective and safe therapy (Gad et al., 2004).

PDT is indicated for the treatment of local diseases caused by microorganisms, being considered a procedure that allows the inactivation and/or destruction of microorganisms by the correlation of three components, a non-toxic chemical photosensitizing agent (PS), a complementary light source and molecules of oxygen. PSs previously incorporated into the cellular structures of microorganisms are capable of absorbing electrons from photon excitation and stimulating a series of reactions involving the formation of free radicals and reactive oxygen species (ROS) providing tissue destruction quickly, after penetrating the cells of microorganisms without damaging surrounding tissues or promoting bacterial resistance (Nemezio et al., 2017).

Currently, PDT is already accepted as effective in the treatment of various oral curative conditions, with the use of antimicrobial photodynamic therapy (aPDT) being cited to control the development/growth of biofilm (Azizi et al., 2016; Nemezio et al., 2017). Several investigations emerged, according to this context, to study aPDT, suggesting the application of this therapy in situations of microbial resistance or in association with existing drugs to increase their effectiveness (Di Poto et al., 2009).

Few systematic reviews have demonstrated the applicability of PDT using MB as a photosensitizing agent against some oral diseases such as caries (De Oliveira et al., 2019), fungal infections (Shen et al., 2020), leukoplakia (Li et al., 2019), in root decontamination in endodontics (Stuber et al., 2021), often with *in vitro* assays.

By acting selectively in the area of application, it does not cause damage to the healthy tissues of the host, without evidence of side effects (Shrestha et al., 2010; Ghorbani et al., 2018; Monteiro et al., 2021) having with inconveniences, some controllable challenges such as the choice of light source, since the laser has a light that concentrates great energy in a small area (Takasaki et al., 2009), which can generate high temperatures causing damage to the tissues surrounding the tooth in the case of endodontic therapies that employ an intra-radicular optical fiber, but the replacement by LED, also with a high dose of energy, will not increase the temperature (Nagata et al., 2012). The possibility of staining the tooth structure, with the use of methylene blue dye, can be another inconvenience, however, by reducing the pre-irradiation time from 10 minutes to 5 minutes, this can be resolved (Ramalho et al., 2017).

As it presents minimal disadvantages, limited unwanted effects and it has a low probability of promoting the development of resistance against microorganisms, PDT has numerous advantages compared to conventional antibiotics and antifungals (Jori et al., 2006; Santezi et al., 2018). Effective in different classes of microorganisms, such as Gram-positive and Gram-negative bacteria and yeasts, having the photosensitizer type as an essential component in the aPDT process (Garcez et al., 2013).

Synthetic and natural pigments can be used as PS for PDT, and they need to have a cationic charge, which allows their rapid union or penetration into bacterial cells, demonstrating that these compounds have a high degree of selectivity to destroy microorganisms, having little toxicity for bacteria mammalian host cells (Fonseca et al., 2008).

Widely used, methylene blue (MB) is a PS phenothiazine indicated for aPDT, having important factors such as its concentration and pH in the practice of these bactericidal applications (Huang et al., 2019). It has low toxicity to human cells, high absorption rate in its wavelength that allows the generation of ROS that are cytotoxic to bacteria (Wainwright, 2005).

PDT acts after the penetration of PS into microorganisms and irradiation by a light source, corresponding to the absorption spectrum of the PS used, favoring the absorption of radiant energy, inducing the production of hydroxyl radicals, peroxides and superoxides, initiating reactions in free radical chain (type I reaction) or higher energy singlet oxygen species (1O_2) that have strong oxidizing properties (type II reaction) (Hamblin, 2016; Fumes et al., 2018).

It appears as an alternative therapeutic modality widely used in several areas of dentistry, such as in periodontics with reduced viability of periodontopathogenic microorganisms (Pimentel & Dias, 2021) *in vitro* (Javali et al., 2019) and *in vivo* (Soareas et al., 2019) studies, and in studies using the diode laser associated with blue of methylene as adjunctive therapy to

basic scaling and root planing *in vivo* treatments (Filipini et al., 2019; Malik & Alkadhi, 2020) demonstrating the efficacy of this therapy; as for carious lesions, this therapy can have preventive action, interfering with the cariogenic biofilm, (Nemezio et al., 2017; Fumes et al., 2018; De Oliveira et al., 2019) in endodontics, the effective of PDT was evaluated *in vitro* (Freitas et al., 2019; Li et al., 2021) with positive results; as well as in the control of microorganisms involved in the development of lesions in the oral mucosa, such as denture stomatitis, candidiasis (Freitas et al., 2017; Daliri et al., 2019) and leukoplakia (Li et al., 2018).

Given the above, this systematic review of the literature aims to identify the scientific production available in the last 5 years regarding the action of PDT with the photosensitizer MB on the microorganisms present in oral biofilm.

2. Methodology

The article was developed as a systematic review and its elaboration took place through the following question: What is the scientific production available on antimicrobial photodynamic therapy (aPDT) for oral decontamination using methylene blue?

The keywords used were influenced by the research objectives, searching in the Descriptors in Health Sciences and being used to identify specific articles on the topic addressed. Among the words, the following were selected in English: Photochemotherapy; Photosensitizer; Mouth; Biofilms; methylene blue; Dental Plaque.

The following phases were used to implement this review: identification of the topic and formulation of the research question, definition of criteria for inclusion and exclusion of studies, establishment of the content to be informed of the selected articles, analysis of studies included in the systematic review, analysis of results and synthesis of knowledge and description of the review (Mendes et al., 2008).

The focus question generated for this study was developed using the PICO strategy, which means Population (indicated as contamination by oral microorganisms), type of Intervention (PDT with methylene blue), Comparison (not applicable) and Result (antimicrobial activity) (Santos et al., 2007).

With using online access, the research was based in: *MEDLINE / Pubmed* (National Library of Medicine, Maryland) and Scopus from Elsevier, with articles being systematically searched for the period from 2017 to 2021.

The inclusion criteria established were studies that presented the use of antimicrobial PDT using methylene blue in oral microorganisms and biofilm, scientific articles published from 2017 to May 2021 and indexed in the databases: *MEDLINE / PubMed* and Scopus, original articles (*in vitro*, *in situ* and *in vivo*).

The exclusion criteria were as follows: literature reviews, cohort studies, non-scientific texts /scientific publications without full text available online reports, editorials, news, duplicates and articles that did not duplicates and articles that did not meet the selected topic, studies not published in English and duplicated works in more than one database.

After the search for publications, carefully following the defined inclusion and exclusion, the selected scientific articles had the title and abstract read exhaustively in order to adapt to the guiding question of this review. Therefore, information regarding the selected articles, such as numerical code, author, study type, design and level of evidence (LE), are described in Table 1.

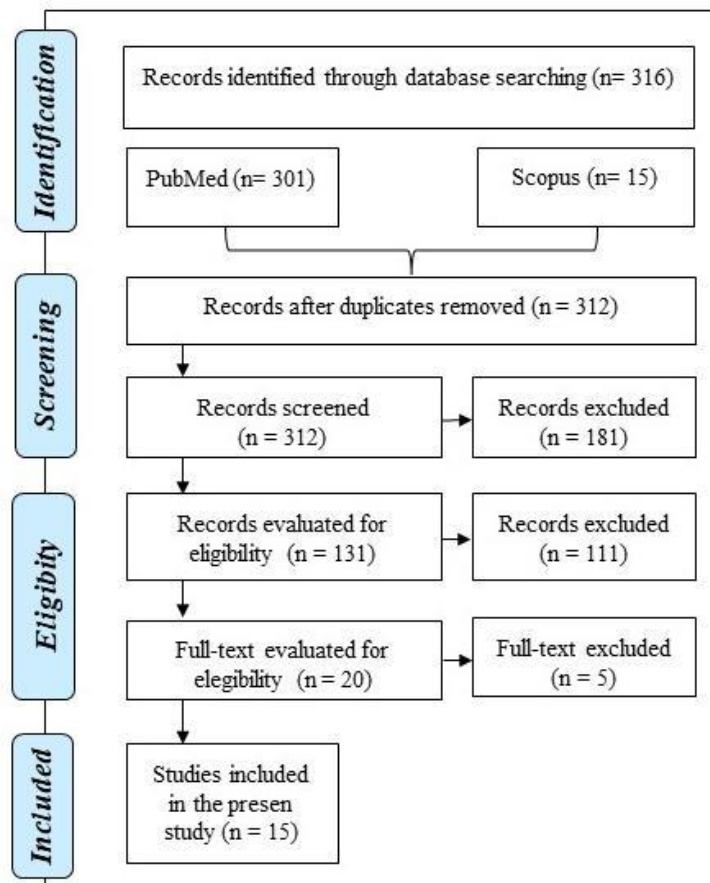
The levels of evidence are classified into: level 1 - Meta-analysis or Systematic Reviews; level 2 - Randomized controlled clinical trial; level 3 - Clinical Trial without Randomization; level 4 - Cohort and Case-Control Studies; level 5- Individual case-control study; Level 6 - Systematic Reviews of Descriptives and Qualitative Studies; level 7 - Descriptive or Qualitative Studies; and level 8 - Expert opinion (Burns; Rohrich; Chung, 2011).

Data extraction was performed by two examiners (L.C.T.M. and K.G.C.F.).

3. Results

Among the studies, 301 articles were identified in the *MEDLINE / PubMed* databases and 15 in Scopus, resulting in 316 scientific articles, of which 181 articles were removed by time interval and 4 by duplication. After the evaluation of titles and abstracts, 111 articles were removed, leaving 20 publications. After reading the full texts of these articles, 5 were excluded, as detailed in Figure 1, below, describing in the flowchart of the filtering process (Figure 1).

Figure 1 - Study selection diagram.



Source: Authors (2021).

Of the 15 articles evaluated, 14% were publications from the year 2017; 14% from 2018; 47% were published in 2019 and 20% were from 2020, 5% were published in 2021. Considering the place of publication, 67% were national, with two being held in Ribeirão Preto, two in São Paulo and two in Araraquara, in the cities of São José dos Campos, Santa Maria, Salvador and São Luís do Maranhão, one in each. As for international studies, 33% of the studies were published, with two publications in the city of Tehran, Iran and one in Abha and one in Riyadh both in Saudi Arabia, and one in Beijing, China. Of the samples used in the studies, 73% of the studies were *in vitro*, 7% were *in vivo*, and 20% used clinical samples (Table 1).

Regarding the levels of evidence, they were level 2 - randomized control clinical trial: 2 studies; level 3 - non-randomized control clinical trial: 1 study; level 5 - individual case-control study: 1 study; level 7 - descriptive or qualitative: 11 studies (Table 1).

Table 1. Summary of selected articles for this review according to numerical code, author (s), type of study, design, and level of evidence (2017-2020) (n=15).

N°	Author (s)	Type of study	Study delimitation	LE*
A1	Freitas <i>et al.</i> [2017]	<i>in vitro</i>	descriptive or qualitative	7
A2	Nemezio <i>et al.</i> [2017]	<i>in vitro</i>	descriptive or qualitative	7
A3	Da Colina <i>et al.</i> [2018]	<i>in vitro</i>	descriptive or qualitative	7
A4	Fumes <i>et al.</i> [2018]	<i>in vitro</i>	descriptive or qualitative	7
A5	Azizi <i>et al.</i> [2019]	<i>in vitro</i>	descriptive or qualitative	7
A6	Freitas <i>et al.</i> [2019]	<i>in vitro</i>	descriptive or qualitative	7
A7	Filipini <i>et al.</i> [2019]	<i>in vivo</i>	randomized control clinical trial	2
A8	Javali <i>et al.</i> [2019]	<i>in vitro</i>	descriptive or qualitative	7
A9	Soares <i>et al.</i> [2019]	Clinical	clinical study without randomization	3
A10	Eduardo <i>et al.</i> [2019]	Clinical	individual case-control study	5
A11	Daliri <i>et al.</i> [2019]	<i>in vitro</i>	descriptive or qualitative	7
A12	Zago <i>et al.</i> [2020]	<i>in vitro</i>	descriptive or qualitative	7
A13	Malik e Alkadhi [2020]	Clinical	randomized control clinical trial	2
A14	Furtado <i>et al.</i> [2020]	<i>in vitro</i>	descriptive or qualitative	7
A15	Li <i>et al.</i> [2021]	<i>in vitro</i>	descriptive or qualitative	7

Legend: *LE: level of evidence. Source: Author (2021).

Considering the light-emitting devices used, the results showed that 66% used Laser (Table 2) while 20% used LED in photodynamic therapy (Table 3). The articles that used the association of two non-ionizing light sources (Laser and LED) represented 14% das publications, and in one of these studies both light sources were used in addition to ultraviolet and infrared (Table 4). The power density used ranged from 0.0026 W/cm² to 1415 W/cm². The energy density ranged from 3 J/cm² to 640 J/cm². The power ranged from 10 mW to 110 mW. The application time ranged from 24 to 294 seconds. The wavelength most used in 87% of the studies was 660 nm (Tables 2, 3 and 4).

Table 2. Study numeric codes and the summary of studies selecty in the sample review, light source type (Laser), power density, energy density, power, application time, wavelength, and result.

Nº	Sample	Light source	Power Density	Energy density	Power output	Application time	Wavelength	Result
A2	<i>Streptococcus mutans</i> biofilms after growing for 48 h, the biofilms were submitted to the following treatments, twice daily (n = 4): (Gr 1) 0.9% NaCl (NaCl) as the negative control; (Gr. 2) 0.12% (CHX) as the positive antibacterial control; (Gr. 3) diode laser combined with methylene blue (MB).	Laser	1415 W/cm ²	320 J/cm ²	100 mW	90 s	660 nm	Positive
A4	The sample consisted of 15 <i>Streptococcus mutans</i> biofilms cultures, randomly divided into five groups and 15 <i>C. albicans</i> cultures, also divided into five groups: 0.12% chlorhexidine digluconate (CHX, positive control); saline solution at 0.89% (NaCl, negative control); antimicrobial photodynamic therapy (aPDT)with Methylene blue, and low-power laser with preirradiationtime of 1 min (aPDT 1 min); 2 min (aPDT 2 min), or 5 min (aPDT 5 min).	Laser	1.66 W/cm ²	640 J/cm ²	100 mW	180 s	660 nm	Positive (<i>S. mutans</i>) Negative (<i>Candida</i>)
A6	Standardized suspensions of <i>Enterococcus faecalis</i> were submitted to sub-inhibitory (PDT) with photosensitizers (PS) chlorin-e6 (Ce6) and methylene blue (MB), combined or not to the antimicrobial peptides (AP): peptide aurein (AU) (monómero de aureína 1.2) ou peptide (AU) ₂ K (dímero C-terminal aurein 1.2), in grupos (Gr): Gr: Control- untreated; Gr: Ce6/PDT, Gr: AU; Gr.: Ce6/PDT + AU; Gr.: (AU) ₂ K; Gr. Ce6-PDT + (AU) ₂ K; Gr: Control untreated; Gr: MB/PDT, Gr: AU; Gr. MB/PDT +AU; Gr. (AU) ₂ K; Gr. MB/PDT + (AU) ₂ K;	Laser	150 mW/cm ²	30 J/cm ²	Not informed	Not informed	664 nm	Positive (Upper Gr. Ce6-PDT + (AU) ₂ K)
		Laser	150 mW/cm ²	45 J/cm ²	Not informed	Not informed	664 nm	Positive
A7	Induce experimental periodontitis in 35 diabetic rats 5 groups: - LG (without treatment, n = 5), NLG- no ligaments and without treatment, n = 5); - SRPG (SRP, n = 10)- Ligate animals, treated with SRP; - aPDTW: (SRP+aPDT-MB/water, n = 10)- Ligate animals, SRP plus aPDT-MB (0.01%) solubilized in ultra-pure water; - aPDTEt:(SRP+aPDTMB/water/ethanol/carboxymethylcellulose, n = 10) Ligate animals; - SRP plus aPDT-MB (0.01%) solubilize in ultra-pure water, ethanol, and carboxymethylcellulose.	Laser	1.07 W/cm ²	29.64 J/cm ²	30 mW	24 s	660 nm	Positive Positive (Upper aPDTEt)
A10	Severe oral infection caused by <i>Pseudomonas aeruginosa</i> in a 37-year-old female patient, under chemotherapy	Laser	2.5 W/cm ²	8 J (1 J per point)	100 mW	80 s	660 nm	Positive

for a recurrent colon adenocarcinoma, reported an intense oral pain and difficulties for mastication and swallowing								
A11	Samples of <i>Candida albicans</i> standard strain in Groups: 15 groups including 13 experimental groups, one positive control group, and one negative control group (a total of 150 samples): 460-nm laser+CUR; Nystatin; 460-nm duty cycle laser+CUR; 460-nm laser; 660-nm/100-mW laser (100 seconds)+0.02% MB; 660-nm/100-mW laser (100 seconds)+0.01% MB; 0.02% MB; 0.01% MB; 660-nm/10-mW laser (60 seconds)+0.01% MB; 660-nm/100-mW laser (100 seconds); 660-nm/10-mW laser (60 seconds); Light-Curing.+CUR; CUR; Negative control; Positive control.	Laser		25 mW	30 s	460 nm	Positive (laser+CUR upper)	
		Laser		100 mW	60 s	660 nm	Positive	
A13	Biofilm (unspecified microorganisms) Patients with gingivitis were randomly divided into 2- groups: - In the test-group, patients underwent MD with adjuvant aPDT; -and in the control-group, patients underwent MD alone.	Laser		0.0125 J/cm ²	150 mW	60 s	660 nm	Positive
A14	A standard suspension of <i>Streptococcus mutans</i> was prepared and submitted at sensitization of (MB) for 0, 1, 3 and 5 min (G1 – G4 groups, respectively) and irradiated with a red laser. A control group using PBS was performed as well (G5-PBS).	Laser		0.0125 J/cm ²	150 mW	60 s	660 nm	Positive
		Laser		321 J/cm ²	100 mW	90 s	660 nm	Positive
A15	Enterococcus faecalis bacteria in planktonic and biofilm forms with potassium iodide (KI) potentiation. Group 1: PBS (negative control group); Group 2: 1.5% NaOCl (positive control group); Group 3: MB (0.4 µM) - PDT; Group 4: MB (0.4 µM) - PDT + KI; Group 5: MB (20 µM) - PDT; Group 6: RB (0.2 µM) - PDT; Group 7: RB (0.2 µM) - PDT + KI; Group 8: RB (10 µM) - PDT.	Laser	60 mW/cm ²	6 J/cm ²		100 s	660 nm	Positive (MB+ KI upper)
		Laser	60 mW/cm ²	6 J/cm ²				565 nm

Legend: mW/cm² (Milliwatts per square centimeter); J/cm² (Joule per square centimeter); s (seconds); nm (nanometer). A2: Nemezio *et al.* (2017): NaCl (sodium chloride), CHX (chlorhexidine digluconate); A4: Fumes *et al.* (2018); A6: Freitas *et al.* (2019): photodynamic therapy (PDT), photosensitizers (PS), chlorin-e6 (Ce6) and methylene blue (MB); A7: Filipini *et al.* (2019): SRP scaling and root planing, aPDT antimicrobial photodynamic therapy, MB methylene blue, Et (ethanol), W (water); A10: Eduardo *et al.* (2019); A11: Daliri *et al.* (2019): CUR: curcumin; MB :azul de metileno; A13:Malik e Alkadhi (2020): aPDT (antimicrobial photodynamic therapy) MD (mechanical debridement); A14: Furtado *et al.* (2020): methylene blue (MB), PBS- buffered saline solution; A15: Li *et al.* (2021): MB- methylene blue, KI-potassium iodide, RB- rose Bengal, NaOCl (Sodium hypochlorite). Source: Author (2021).

Table 3. Study numeric codes and the summary of studies selecty in the sample review, light source type (LED), power density, energy density, power, application time, wavelength, and result.

Nº	Sample	Light source	Power Density	Energy density	Power output	Application time	Wavelength	Result
A3	<i>Candida albicans</i> biofilm and planktons foram submetidas a PDT nos seguintes grupos: MB 2 - PS = MB 20 mg / L in saline solution, in planktons MB 5 - PS = MB 50 mg / L in saline solution, in biofilm MB 2 - OF = MB 20 mg / L in the oral formulation; MB 5 - OF = MB 50 mg / L in the oral formulation; PS = saline solution - control group (NaCl 0.9%).	LED	2.6 mW/cm ²	4.7 J/cm ²	100 mW	600 s	640 nm	Positive
								Positive
								Positive
								Positive (Gr. MB 5 - OF upper)
A9	Twenty-one patients in the study. Three biofilm collections were performed around the brackets and gums of the inferior central incisors; first before any intervention (Control); second after 5min of pre-irradiation and the last one immediately after AmPDT Group: Control group; Photosensitizing Group; and PDT group.	LED		30 J/cm ²	110 mW	294 s	640 nm	Positive
A12	Periodontal disease (unspecified microorganisms) in the planktonic and biofilm phase. Groups (Gr.) without treatment (NT), and control of light (Light); and aPDT-PS (PS-photosensitizers): Gr.1PS; Gr.2aPDT (PS methylene blue); Gr.3PS; Gr.4aPDT (PS chlorin-e6); Gr.5PS; Gr.6aPDT (PS Curcumin).	LED	171 mW/cm ²	120 J/cm ²		60 s	660 nm	Positive
		LED	171 mW/cm ²	30 J/cm ²		120 s	660 nm	Positive
		LED	154 mW/cm ²	30 J/cm ²		180 s	450 nm	Positive (upper CUR)

Legend: mW/cm² (Milliwatts per square centimeter); J/cm² (Joule per square centimeter); s (seconds); nm (nanometer). A3: Da Colina *et al.* (2018); OF = sodium dodecyl sulfate, oral formulation without MB; MB: methylene blue; A9: Soares *et al.* (2019); AmPDT (antimicrobial photodynamic therapy); A12: Zago *et al.* (2020); Photosensitizers (PS), chlorin-e6 (PS), Curcumin (PS). Source: Author (2021).

Table 4. Study numeric codes and the summary of studies selecty in the sample review, light source type (Laser in comparison to LED), power density, energy density, power, application time, wavelength, and result.

Nº	Sample	Light source	Power Density	Energy density	Power output	Application time	Wavelength	Result
A1	<i>Candida glabrata</i> biofilm PDT sessions on polymerized acrylic resin specimens formed after 5 days PDT at 24 h intervals: (1) control group (untreated); (2) group PDT-MB+laser; (3) group PDT-Ery+LED and one application PDT (Gr. 2 e Gr. 3); (4) group PDT-MB+laser; (5) group PDT-Ery+LED and four applications (Gr. 4 e 5);	Laser	92 mW/cm ²	26.3 J/cm ²	35 mW	285 s	660 nm	Positive
		LED	237 mW/cm ²	42.63 J/cm ²	90 mW	180 s	532 nm	Positive (Group 5- upper)
A5	Samples of <i>Streptococcus mutans</i> were prepared in 110 test tubes and were randomly assigned to 11 groups after colony counting: 1) Positive control group, 2) Negative control group, 3) CUR extract group, 4) 460-nm laser group, 5) 460-nm continuous laser+CUR group, 6) 460- nm discontinues 50% duty cycle laser+CUR group, 7) 660-nm laser group, 8) 660-nm laser+MB group, 9) MB group, 10) dental light-curing group, 11) chlorhexidine (CHX) group.	Laser			100 mW	60 s	460 nm	Positive (Upper Gr 5)
		LED			100 mW	100 s	660 nm	Positive
A8	Four bacterial strains of oral biofilm were isolated from the sample of patients divided into: Group 1. Control – untreated; Group 2. Light alone – bacterial suspensions radiated with varying wavelengths; Group 3. MB alone – methylene blue was added to each sample to a final concentration of 0.01% weight/volume; Group 4. MB and light based technologies – bacterial samples were added with photosensitizer as in group 3 and then subsequently treated with light of varying wavelength as in group 2; Group 5. TiO ₂ and light-based technologies – bacterial samples were added with TiO ₂ and then subsequently treated with light of varying wavelength as in group 2.	LED				60 s	315-400 nm	Positive
		LED				60 s	180-300 nm	Positive
		Laser		13J, 18 J e 30 J		60 s	660 nm	Positive (Gr. 5-upper)
		LED				60 s	430-480 nm	Positive
		Laser		321 J/cm ²	100 mW	90 s	660 nm	Positive

Legend: mW/cm² (Milliwatts per square centimeter); J/cm² (Joule per square centimeter); s (seconds); nm (nanometer). A1: Freitas *et al.* (2017); MB (methylene blue), Ery (erythrosine); A5: Azizi *et al.* (2019); CUR (curcumin); MB (methylene blue), CHX (chlorhexidine); A8: Javali *et al.* (2019); MB methylene blue, TiO₂ (titanium dioxide). Source: Author (2021).

Importantly, the effectiveness of PDT is influenced by factors such as the light dose administered to target cells and the time of light exposure (Kwiatkowski et al., 2018). Considering these factors, it is possible to observe in the Tables (2 and 4) that the LASER was the most used light source, corroborating other systematic reviews (Fumes et al., 2018), which justify the use of the Low power laser due to the advantages over other types of light sources, because they have a narrow spectrum that allows a more specific interaction with the photosensitizers (Bevilacqua et al., 2007; Kwiatkowski et al., 2018).

In this as in other systematic reviews, the wavelength often used was 660 nm (Pereira et al., 2011; Diniz et al., 2015; Queiroz et al., 2015; Borsatto et al., 2016; De Oliveira et al., 2019) the choice of wavelength should be the closest absorption peak of the photosensitizer (Wainwright, 2000). The choice of light device must be between the absorption peaks of Methylene Blue (λ 598 - 664nm) (Simmons et al., 2015). The use of inappropriate wavelength reduces or even does not photoactivate the photosensitizer making it thus PDT is not effective (Soares et al., 2019).

The selected articles chose the following samples of microorganisms: three studies were identified using only *Candida* species (two with *Candida albicans* and one study with samples of *Candida glabrata*) and three studies using samples of *Streptococcus mutans*. One study used samples of *Candida albicans* or samples of *Streptococcus mutans*, and another study used strains of *Enterococcus faecalis*, *Staphylococcus aureus* or *Enterococcus faecium*. *Pseudomonas aeruginosa* samples were identified in one study, as well as *Enterococcus faecalis* samples were used in another study. Two studies used microorganisms related to periodontal disease, but not specified and three studies used oral biofilm and among them only one isolated the strains: *Aggregatibacter actinomycetemcomitans* (Aa), *Fusobacterium nucleatum* (Fn), *Porphyromonas gingivalis* (Pg) and *Treponema denticola* (Td) (Table 5).

Table 5. Microorganisms evaluated; type of photosensitizer used concentrations, new formulations, and properties.

N°	Microorganisms	photosensitizer used concentrations	pre-irradiation time	new formulation	properties
A1	<i>Candida glabrata</i>	MB 300 µM = And erythrosine of 400 µM	600 s		antimicrobial effects
A2	<i>Streptococcus mutans</i>	MB 0.01%	300 s		antimicrobial effects
A3	<i>Candida albicans</i>	MB 100 mg/L= 0.01% 50 mg/L = 0.005% 20 mg/L = 0.002% 10 mg/L= 0.001%	300 s	MB+ sodium dodecyl sulfate 0.25% - SDS and urea 1 mol/L)	antimicrobial action and new formulations
A4	<i>Streptococcus mutans</i> or <i>Candida albicans</i>	MB 0.01%	60 s, 120 s, 180 s		antimicrobial effects
A5	<i>Streptococcus mutans</i>	MB 0.02% CUR 10.2 %	Not informed		antimicrobial effects
A6	<i>Enterococcus faecalis</i> or <i>Staphylococcus aureus</i> or <i>Enterococcus faecium</i>	MB 78 µM Ce6 chlorin e-6 = 42 µM Peptides (AU) =16 µM or (AU) ₂ K = 25 µM	Not informed	MB + (AU); MB + (AU) ₂ K	antimicrobial action and new formulations
A7	Periodontal disease (unspecified microorganisms)	MB 0.01%	60 s	MB + solubilized in ultra-pure water, ethanol, and carboxymethylcellulose	antimicrobial effects and new formulations
A8	Biofilm and isolated strains: <i>Aggregatibacter actinomycetemcomitans</i> (A.a), <i>Fusobacterium nucleatum</i> (F.n), <i>Porphyromonas gingivalis</i> (P.g) and <i>Treponema denticola</i> (T.d).	MB 0.01% titanium dioxide (TiO ₂)	Not informed		antimicrobial effects

A9	Biofilm formed by microorganisms gram-negative and gram-positive.	(MB +Toluidine Blue, 1: 1, 12.5 µg/mL) = 0.00125%	300 s	Methylene Blue + Toluidine Blue	antimicrobial effects new formulations
A10	<i>Pseudomonas aeruginosa</i>	MB 0.01%	300 s		antimicrobial effects
A11	<i>Candida albicans</i>	MB 0.01% and 0.02% CUR 10.2%	Not informed		antimicrobial effects
A12	Periodontal disease (unspecified microorganisms)	MB 25 µg/mL = 0.0025%; 50 µg/mL = 0.005%; 75 µg/mL = 0.0075%; 100 µg/mL = 0.01%	600 s		antimicrobial effects
A13	Biofilm (unspecified microorganisms)	MB 400 µg/mL = 0.04%	15s		antimicrobial effects
A14	<i>Streptococcus mutans</i>	MB 0.005%	60 s, 180 s e 300 s		antimicrobial effects
A15	<i>Enterococcus faecalis</i>	MB 20 µM MB 0.4 µM	600 s		antimicrobial effects new formulations

Legend: MB (methylene blue); CUR (curcumin); s (seconds). A1: Freitas *et al.* [19], A2: Nemezio *et al.* [3], A3: Da Colina *et al.* [20], A4: Fumes *et al.* [13], A5: Azizi *et al.* [21], A6: Freitas *et al.* [22], A7: Filipini *et al.* [23], A8: Javali *et al.* [24], A9: Soares *et al.* [25], A10: Eduardo *et al.* [26], A11: Daliri *et al.* [27], A12: Zago *et al.* [28], A13: Malik e Alkadhi [29], A14: Furtado *et al.* [30], A15: Li *et al.* [31], Source: Author (2021).

The concentrations used in the photosensitizer described in table 5 ranged from 0.001% to 0.04% (values shown in the table), and the most used, in 33% of the studies, was 0.01%, followed by a concentration of 0.005% in 20% of the studies. In relation to the pre-irradiation times, the results showed that they ranged from 15 to 600 seconds, with the time of 300 seconds being the most used in 40% of the studies. Most studies used MB, however 25% of the studies associated MB with other products analyzing new formulations (Table 5). The concentrations used in the photosensitizer ranged from 0.001% to 0.04% (values shown in the table), and the most used, in 33% of the studies, was 0.01%, followed by a concentration of 0.005% in 20% of the studies. In relation to the pre-irradiation times, the results showed that they ranged from 15 to 600 seconds, with the time of 300 seconds being the most used in 40% of the studies. Most studies used MB, however 25% of the studies associated MB with other products analyzing new formulations (Table 5).

The use of MB can cause staining in the dental structure, and this change can be minimized by reducing the pre-irradiation time from 10 minutes to 5 minutes (Ramalho et al., 2017). In the Table, it was identified that the pre-irradiation time widely used was 5 minutes, corroborating other systematic reviews found in the literature (Fumes et al., 2018; De Oliveira et al., 2019;). The pre-irradiation time is a fundamental factor for the diffusion of PS through the biofilm (Guglielmi et al., 2011). Authors suggest that the longer the pre-irradiation time, the more the dye will penetrate the dental biofilm (Fumes et al., 2018). However, there is still no protocol defined regarding the pre-irradiation time for this therapy to be effective. Table 5 shows that the studies presented used different pre-irradiation times, with a predominance of 5 minutes for both planktonic microorganisms (Colina et al. 2018; Eduardo et al., 2019; Furtado et al., 2020) and biofilm (Nemezio et al., 2017; Colina et al. 2018; Soares et al., 2019).

4. Discussion

There is a growing demand to find effective treatment modalities for bacterial elimination with few side effects and bacterial resistance, and the use of aPDT has been considered an alternative for several therapeutic modalities (Santezi et al., 2018; Huang et al., 2019; Zago et al., 2020). Is based on the principle that a photoactive dye, also called a photosensitizer, binds to a target cell, and is activated by a specific wavelength of light. In this process, Reactive Oxygen Species (ROS) such as singlet oxygen and free radicals are formed, producing toxicity to the cell without damage to the host tissues (Jori et al., 2006; Warriar et al., 2021.).

In the present review, the studies corroborated the antimicrobial efficacy of photodynamic therapy in oral biofilm, using protocols that are slightly different from each other, however demonstrating the effectiveness of this therapy both with the single use of methylene blue and in some associations.

Widely used in PDT, the Methylene Blue (MB) has action mechanisms (Type I or Type II) defined by its aggregation state (stage that modulates the type of photochemical reaction) (Junqueira et al., 2000; Prochnow et al., 2016). *In vitro* research has emphasized that the vehicle in which MB is involved has a significant influence on the phototoxic effect of PDT, as well as this vehicle can affect the outcome of this reaction generating lower reactive oxygen species and singlet oxygen production (George & Kishen, 2007), with reduced half-life (Meisel & Kocher, 2005) and low diffusion potential (Ochsner, 1997). Since the use of new oral formulations (OF) associating MB to sodium dodecyl sulfate 0.25% - SDS and urea 1 mol / (a3) and ethanol, respectively, minimize these alterations, thus being able to stimulate the emergence of new formulations for clinical applications (Colina et al., 2018; Filipini et al., 2019).

Another important factor for the effectiveness of PDT is that the definition of photosensitizers (PS) must consider the target cell, since gram-positive bacteria present structural differences in relation to gram-negative, therefore, they have different mechanisms of interaction with the drugs. Phenothiazines have an intrinsic cationic charge and an association of MB and toluidine blue makes them more effective against many bacteria, providing a greater reduction in the number of

microorganisms. PDT associated with MB can be used for root canal decontamination, however such products have problems associated with dentin staining (Ramalho et al., 2017), and an association of MB with potassium iodide (KI) salt eliminated this alteration and may have been a viable option to the usual methods of disinfection in endodontic therapy (Li et al., 2021).

However, further investigations are needed with *in vivo* assays with new associations in order to define safe concentrations and vehicles to support clinical protocols.

This therapy was recently proposed to combat clinically relevant biofilms, such as dental biofilms, ventilator-associated pneumonia, chronic wound infections, and oral candidiasis among other microorganisms (Hu et al., 2018). The *in vitro* and *in vivo* studies described in this review demonstrated the eradication of the biofilm or its substantial reduction when using aPDT. ROS are produced upon photoactivation and attack surrounding targets, including proteins, lipids, and nucleic acids arranged in the biofilm matrix, on the cell surface, and within microbial cells. ROS induce multiple and non-specific damage to cells promoting the destruction of both planktonic cells and biofilms (Hu et al., 2018).

According to Freitas et al. (2019) this biome, when structured, the extracellular polymeric matrix self-produced by the biofilm protects them and makes their elimination difficult, making the inhibition of the formation of these structures the key to the successful treatment of a biofilm infection. Thus, in their study, Freitas et al., (2019), combined aPDT and antimicrobial peptides, representing a new approach as antibiofilm strategies, aiming to associate antimicrobial peptides (AMPs), defined as oligopeptides with a broad spectrum of action against microorganisms, and these AMPs, aurein 1.2 monomer (AU) and the C-terminal aurein 1.2 (AU)2K dimer were associated with aPDT to inhibit the biofilm formation process by *Enterococcus faecalis*. To analyze the ability of each treatment to inhibit biofilm formation the viability of bacteria that were able to adhere to the bottom of the well was evaluated, that is, that continued the process of biofilm formation (adhesion and protection).

The study described above used methylene blue (MB) and chlorin-e6 (Ce6) as PS, as they have different structures and chemical characteristics and interact differently with cells, being activated by the 664 nm diode laser, varying the density of energy for MB at 45 J/cm² and for Ce6 30 J/cm² with a power density of 150 mW/cm², and all irradiated treatments used successfully inhibited biofilm growth. Treatment of Ce6-PDT and Ce6-PDT + (AU) 2K groups were effective in reducing biofilm in the initial stage, showing reductions ranging from 95.5% (Ce6-PDT + (AU) 2K) to 78% (Ce6 -PDT) compared to the control. Using of MB-PDT + (AU) inhibited almost 60% of the biofilm compared to the control group, however the (AU) 2K dimer provided a significant decrease of more than 70% in cell viability alone, almost completely inhibiting the formation of biofilms when combined with MB-PDT or Ce6-PDT groups.

Likewise, the case report by Eduardo et al. (2019) described in this review, presented positive clinical outcomes after some inactivation sessions with antimicrobial photodynamic therapy mediated by methylene blue dye, describing the clinical case of an oncological patient with lesions oral infections caused by *Pseudomonas aeruginosa* (*P. aeruginosa*), refractory to antibiotic treatment, using the laser with a wavelength of 660 nm, 100 mW, 8 J being 1 J per point. *P. aeruginosa* is a gram-negative bacterium present in nosocomial infections with high morbidity and mortality, being a microorganism often resistant to antibiotics, leading to clinical complications. The intraoral lesion described in this report had multiple and extensive necrotic areas in the gingiva of the superior alveolar crest, being treated with systemic antibiotic therapy for 4 days and, due to the maintenance of the gingival necrotic areas, aPDT was used for 3 weeks in these lesions, which culminated in with complete remission of the lesions. The authors reinforced that treatment with aPDT adjuvant to systemic antibiotics eliminated *P. aeruginosa* infection in the oral cavity and avoided invasive interventions (Eduardo et al., 2019).

Some combined treatment protocols employing PDT and antibiotics such as the one addressed in the case report by Eduardo et al. (2019) as well as the one employed by Freitas et al. (2019) associating aPDT and antimicrobial peptides have the potential to improve the treatment of oral infections in hospital settings and dentistry via eradication of *E. faecalis*, *Enterococcus faecium*, *Staphylococcus aureus* and *P. aeruginosa*, species that are associated with nosocomial and endodontic

infections, directly related to the formation of biofilms on various surfaces biofilms on various surfaces on several surfaces, being highly tolerant and resistant to drugs antimicrobials, also contributing to damage to medical equipment (catheters, prostheses, and valves).

The aPDT plays an effective antimicrobial role, however some independent factors related to the antimicrobial effect of this procedure need to be demonstrated separately before clinical applications. Factors such as photosensitizer concentration, irradiation time (light dose), type of laser and bacterial species can influence the antimicrobial effect of therapy (Huang et al., 2019).

Articles cited in this review presented *in vitro* studies similar to those by Fumes et al. (2018), in which they analyzed different pre-irradiation times of photosensitizers in PDT and their effects on biophimes produced by *Streptococcus mutans* and *Candida albicans*. This drug pre-irradiation time, consisting of the time the dye encounters the biofilm before light application, is an important factor for the diffusion of the photosensitizer through the biofilm (Fumes et al., 2018; Furtado et al., 2020).

Fumes et al. (2018) had as study factors aPDT and methylene blue (0.01%) used in association with diode laser (InGaAlP) with a wavelength of 660 nm, in samples from 15 biofilm cultures of *S. mutans* and 15 cultures of *C. albicans*, both randomly divided into five groups, with chlorhexidine digluconate (0.12% CHX) and saline as positive and negative controls, respectively. The pre-irradiation times of 1, 2 and 5 minutes were used in the photosensitizers, and the results found for the treatment with aPDT, regardless of the pre-irradiation time evaluated, were the same as for the treatment with CHX and when compared to saline solution both were more effective in reducing biofilm. For the *C. albicans* group, there was no statistical difference between the groups. In this way, a pre-irradiation time of 1 minute can be used to reduce the microbial load of *S. mutans*.

In a more recent study, Furtado et al. (2020) employed aPDT *in vitro* with 0.005% methylene blue sensitization for the following pre-irradiation times 0, 1, 3 and 5 minutes as antimicrobial activity against suspensions of *Streptococcus mutans*, divided into 5 groups, being irradiated with red laser (660 nm; 321 J/cm²; 9 J). The tested aPDT groups achieved a significant bacterial reduction in planktonic cultures compared to the control, considering the pre-irradiation times tested, there were no statistical differences between them. Authors reinforced the need for pre-irradiation time, however, a reduction in this time effectively implies a shorter clinical time in the approaches without compromising the antimicrobial effect of the therapy in the *in vitro* parameters used (Furtado et al., 2020).

The aPDT is a multi-stage procedure, including topical administration of the photosensitizer (non-toxic dye), irradiation of visible light, and interaction of the excited state with ambient oxygen to produce ROS (Hu et al., 2018; Freitas et al., 2019; Eduardo et al., 2019). A study by Da Colina et al. (2018) used MB in different vehicles (water, saline solution) that can influence aggregation and associated with an oral formulation (OF) containing MB and SDS (0.25% sodium dodecyl sulfate - SDS and urea 1 mol/L), to be used in clinical aPDT procedures. As it is widely used in antimicrobial photodynamic therapy, MB can define the mechanisms of action (Type I or Type II) by virtue of its state of aggregation, it is essential to identify the relationships between aggregation, mechanisms of action and effectiveness against microorganisms, as well as the possibility of means and formulations that promote more effective mechanisms (Da Colina et al., 2018).

The authors mentioned above emphasized that the use of MB should be considered an important factor for the effectiveness of the therapy, since, depending on the physical-chemical condition of the region in which it is applied, MB can aggregate, and its state of aggregation (monomers or dimers) influences the type of photochemical reaction that occurs, the thus affecting the success of therapy. MB at 20 mg/L and its different vehicles were tested in planktonic culture of *C. albicans*, and the OF tested on biofilms at 50 mg/L MB was necessary to achieve some reduction in cell viability in the biofilm. The parameters followed were the use of 4.68 J/cm² of 640 ± 12 nm LED for the irradiations. Considering the results, for the

authors, it is possible to affirm that, when in the form of monomers, MB was more effective and that the Type II reactions (via singlet oxygen) were the photochemical mechanisms that induced the death of *C. albicans* more efficiently. The research showed valuable information, which can be used in the destruction of microorganisms, employing photodynamic therapy in clinical applications. However, more research is needed to adjust these light parameters for *in vivo* applications (Da Colina et al., 2018).

Several etiological factors can destabilize the natural balance of the oral microbiota and favor the development of infection, among them nutritional deficiencies, the use of oral prostheses, immunosuppression, mucosal lesions, deficiencies in oral hygiene (Siqueira & Fungi, 2004). Studies have suggested that photodynamic therapy can be used as a complement for dental stomatitis, a common disease that affects denture wearers and is characterized by inflammation and erythema in the regions of the oral mucosa that are covered by the denture. In denture wearers, the prevalence of *C. albicans* increases up to 100%, which can be explained by the fact that dentures reduce the flow of oxygen and saliva to the underlying tissue which favors yeast overgrowth (Gleiznys et al., 2015; Freitas et al., 2017).

A study conducted by Freitas et al. (2017), analyzed the effects of one and several sessions of PDT on biofilm of a species of *C. glabrata*, formed in polymerized acrylic resins formed after 5 days. An application of PDT (with laser and LED and their respective PSs) was performed and compared with four sessions of PDT on biofilms at 24-hour intervals (days 6-9). The authors included two types of PDT, with laser irradiation and methylene blue or light-emitting diode (LED) and erythrosine. Comparing treatments, the therapies used in this article (association of laser and methylene blue or LED and erythrosine) were able to reduce *C. glabrata* biofilm, with repeated applications showing greater antimicrobial activity compared to a single session (Freitas et al., 2017).

Considering the protocols deployed in this study by Freitas et al. (2017), LED and erythrosine were more efficient compared to laser-mediated PDT. Furthermore, the higher application power density of LED used in this article compared to laser (92 mW/cm² and 237 mW/cm², respectively), may have promoted the advantage in the LED protocol and, consequently, the better results.

Javali et al. (2019) considered that the incomplete eradication of bacteria from plaque in biofilm retention regions and the growing inconvenience of antibiotic resistance stimulated the emergence of new antimicrobial strategies to favor satisfactory results and minimize antibiotic resistance. The research *in vitro* aimed to analyze the bactericidal activity of a new light therapy and assess the vulnerability of oral plaque microorganisms against light-based technologies with and without the addition of MB dye and light sources used between 180 and 480 nanometers.

In the above study, four bacterial strains from the biofilm dental sample collected from a patient (*Aggregatibacter actinomycetemcomitans*, *Fusobacterium nucleatum*, *Porphyromonas gingivalis* and *Treponema denticola*) were isolated. The groups demonstrated a good antimicrobial activity with a significant decrease of colony forming units (CFU) count compared to the control group using light and PDT based technologies. In all groups tested the antimicrobial activity had high clinical significance with an elimination rate of over 75%. The cited study reinforced the effectiveness of light-based technologies and PDT in eliminating oral bacterial colonization, with a positive impact on oral biofilm. Therefore, it is necessary to explore further the effectiveness of various light sources in the oral cavity for further support of *in vivo* clinical studies (Javali et al., 2019).

Nemezzio et al. (2017) reported the use of aPDT to control biofilm, and highlighted that its effectiveness involves several factors, including the biofilm model used. It aimed to associate the MB with the diode laser in order to verify if this exposure would damage the bacterial viability and would affect the polysaccharide content in an *in vitro* cariogenic biofilm model of *Streptococcus mutans*, simulating oral clinical conditions with episodes of excess and reduction of exposure of sucrose, frequent in the oral cavity. The results found, considering the experimental conditions of evaluation, showed that the

use of laser associated with MB twice a day effectively reduced both the concentration of intra and extracellular polysaccharides as well as the variability of *S. mutans* biofilms. Authors stressed that further studies that develop models of *in vitro* systems that simulate *in vivo* conditions of the oral cavity are essential, especially regarding the presence of dual or multispecies biofilms, biofilms at different stages of maturation and saliva in continuous flow (Nemezzio et al., 2017).

The use of PDT as local therapy for infections it is a recent discovery and its clinical applications are still under development (Da Colina et al., 2018). Several studies have reported the effectiveness of methylene blue as an *in vitro* photosensitizer. However, the literature has only a few clinical trials that showed the applicability of the parameters that are used *in vitro* and *in vivo* studies of an aPDT with MB (Maciel et al., 2016).

In one of the studies in this review, Filipini et al. (2019) evaluated the effect of an experimental formulation of MB (plus 20% ethanol) on aPDT, using it as an adjuvant to scale and root planing (SRPG) in the periodontal treatment of rats diabetics. Forty male Wistar rats received intraperitoneal injections of streptozotocin to induce diabetes, and 35 animals received ligature in the lower right first molar to induce periodontitis. The animals were divided into LNG Group (without ligature); LG groups (ligation and no treatment, n = 5), SRPG root scaling, n = 10), aPDTW (SRP + aPDT-MB / water, n = 10), and aPDTEt (SRP + aPDT-MB / water / ethanol / carboxymethylcellulose, n=10).

The animals were sacrificed after 7 days to evaluate periodontal healing following some parameters such as bone loss area (BL), degree of cellular inflammatory response and percentages of collagen fibers were statistically analyzed. In relation to LG, aPDTEt, aPDTW and SRPG recovered the equivalent of 80%, 46% and 20% of the BL. aPDTEt showed statistically higher collagen content than SRPG and LG and showed higher mean values than LNG ($p>0.05$). The results presented showed that aPDTEt showed promising results. The PDT with the use of the MB /ethanol may have potential as an adjunctive periodontal treatment in diabetics. Authors suggest the association of aPDT provides satisfactory benefits compared to SRPG alone (Filipini et al., 2019).

Similar research carried out *in vivo* in patients undergoing orthodontic treatment reinforced the citations of the above study, regarding the control of biofilm and the prevention of periodontal diseases with the use of aPDT. Orthodontic appliances are used in the treatment of dental and skeletal malocclusions; however, they can make oral hygiene difficult, making patients prone to developing periodontal diseases and caries. In a national study by Soares et al. (2019), the authors warned of the use of orthodontic brackets as a predisposing factor and biofilm alteration, favoring the development of gingivitis, due to the difficulty of cleaning these regions with a toothbrush and periodontal curette, causing accumulation of bacterial plaque and worsening inflammation, in addition to providing gingival hyperplasia usually associated with lack of hygiene.

Soares et al. (2019) considered the use of aPDT as a viable option for the control of these periodontal changes, which can be prevented with antimicrobial agents, however these can lead to increased antimicrobial selectivity to available drugs, an adverse effect that does not exist in photodynamic therapy. Through the use of the phenothiazine compound (methylene blue + toluidine blue) as a photosensitizer, the authors aimed to identify, through the reduction of the number of CFCs, the effectiveness of PDT associated with red LED irradiation ($\lambda 640 \pm 5 \text{ nm}$) in 21 orthodontic patient. They performed three collections of biofilm around the brackets and gums of the lower central incisors, one before any intervention (Control); the second after 5 minutes of pre-irradiation and the last immediately after antimicrobial photodynamic therapy (aPDT). After 24 hours of the growth period of the microorganisms, the CFU count was performed, and the results showed that the aPDT was able to reduce the CFU count by about 90% when compared to the control, and also between the aPDT and photosensitizer. Therefore, aPDT associated with the use of phenothiazine compounds and red LED significantly reduced the number of CFUs in orthodontic patients in the non-randomized crossover clinical trial. Authors reinforced that it is important to use the wavelength closest to the photosensitizer absorption peak for photoactivation to occur. In the present study, the LED device

was emitted at $\lambda 640 \pm 5\text{nm}$, placed between the absorption spectra of methylene blue ($\lambda 664\text{ nm}$) and toluidine blue ($\lambda 626\text{ nm}$) (Soares et al., 2019).

Orthodontic treatments can alter the status of the oral cavity, increasing the colonization of opportunistic microbes (including fungi, predominantly *C. albicans* in oral biofilm) (Perkowski et al., 2019). Oral yeasts are potential pathogens, and regardless of whether they are from the commensal oral flora, they can promote oral inflammatory conditions such as candidiasis (Bandara; Matsubara; Samaranayake, 2017).

Conducted in Saudi Arabia by Malik & Alkadhi (2020), an *in vivo* study with 18 adolescent patients undergoing orthodontic treatment and with gingivitis associated with biofilm accumulation, evaluated the effectiveness of mechanical debridement (DM) and antimicrobial photodynamic therapy against oral biofilm in these patients. Patients were randomly divided into 2 groups aPDT was associated with MB at a concentration of $400\ \mu\text{g/mL}$ and was applied to the mouth/gingival sulcus of all teeth and left in place for 15 seconds. The dye was then irradiated with a continuous wave diode laser (660 nm) at a power of 150 milliwatts for 60 seconds per spot. In the test group, patients underwent DM with adjuvant aPDT; and in the control group, patients underwent MD alone, with 6-month follow-up, being assessed by demographic questionnaires and a gingival index applied at baseline and after 6-month follow-up. In the test and control groups, the gingival index of Teichert et al. (2002) was recorded and measured in 6 regions of the teeth (mesiobuccal, buccal, distobuccal, distolingual/distopalatal, lingual/palatal and mesiolingual/mesiopalatal) per tooth on all upper and lower teeth using a sterile probe.

In the present investigation by Malik & Alkadhi (2020) no participant was diagnosed with oral candidiasis, however, in the test group, oral yeast CFU/ml counts were significantly higher at baseline compared to 6-month follow-up ($p < 0.05$). In the control group, there was no statistically significant difference in the CFU/mL of oral yeasts at baseline and at 6-month follow-up. However, aPDT is a useful adjunct to mechanical debridement in decreasing oral yeast counts among adolescents undergoing orthodontic treatment. In terms of classification, DM with and without aPDT provided the reduction in the GI of adolescents undergoing orthodontic treatment (Malik & Alkadhi, 2020).

Malik and Alkadhi (2020) reinforced the need to consider factors such as photosensitizer concentration separately before *in vivo* applications. In the present study, MB was used at a concentration of $400\ \mu\text{g/mL}$, based on studies by Teichert et al. (2002), who investigated the efficiency of MB-mediated aPDT to treat oral candidiasis in immunosuppressed rats in a model simulating oral clinical conditions, having used MB in different concentrations (MB solution at 250, 275, 300, 350, 400, 450 or $500\ \mu\text{g/mL}$). Teichert et al. (2002) demonstrated that MB at concentrations up to $300\ \mu\text{g/mL}$ significantly reduced oral yeast counts, however, when MB was used in concentrations of at least $400\ \mu\text{g/mL}$, oral yeasts were completely eliminated from the oral cavity of the rats. These results reinforced the proposition that during aPDT, MB would act in a dose-dependent manner (Teichert et al., 2002).

The biofilm describes a sessile community derived from diverse microorganisms, with its cells firmly attached to the substrate and incorporated into the extracellular polymeric matrix, accounting for up to 80% of all bacterial and fungal infections in humans (Hu et al., 2018).

Azizi et al. (2019) investigated the effect of photodynamic therapy comparing curcumin (CUR) and methylene blue (MB) photosensitizers in *S. mutans*, a bacterium of great clinical significance, that colonizes the mouth and is often related to dental caries and diseases and comprises up to 70% of the bacteria in dental plaque. In this experimental *in vitro*, 110 samples were prepared and randomly assigned to 11 groups: 1) Positive control group, 2) Negative control group, 3) CUR extract group, 4) 460 nm laser group, 5) 460 nm continuous laser + CUR group, 6) 460 nm intermittent laser with 50% duty cycle (DC) + CUR group, 7) 660 nm laser group, 8) 660 nm laser group + MB-0.02%, 9) MB group, 10) light-curing group and 11) chlorhexidine (CHX) group. After the interventions, the CFU counts were performed, the results showed that CHX and continuous low power laser of 460 nm + CUR had the highest and most significant effect in inhibiting the growth of bacterial

colonies of *S. mutans* showing significant differences with other groups ($p < 0.001$). The antimicrobial property associated with photosensitizers was proven as both groups had a significant difference with the control group ($p < 0.05$), but there was no significant difference between the two groups in pairwise comparisons and they performed relatively similarly ($p > 0.05$). Therefore, laser irradiation of 460 nm and 660 nm in combination with different photosensitizers promoted a significant reduction of bacterial colonies, however, the CUR group + continuous laser of 460 nm provided greater antimicrobial effect compared to the positive control group ($p < 0.0001$). According to the results, PDT mediated by MB (and red laser) and CUR (and blue laser) can significantly eradicate *S. mutans* colonies. Authors have pointed out that achieving an effective modality of aPDT with few adverse effects is important, however, this research was carried out *in vitro*, and it should be noted that there are significant differences between clinical and laboratory conditions (Azizi et al., 2019).

Considering the high prevalence of candidiasis, caused by the opportunistic yeast *Candida albicans*, present in 80% of oral lesions, as well as drug toxicity antifungal agents and the fungistatic property that favors the emergence of species resistant to drug therapy (Azizi et al., 2016), a study was carried out by Daliri et al. (2019) that aimed of verifying the success of PDT with different photosensitizers such as curcumin (CUR) and methylene blue (MB) and lasers with different parameters, exposed to colonies of *C. albicans*. In this experimental *in vitro*, 150 samples of the standard strain of *C. albicans* were examined using different combinations of CUR and MB photosensitizers with and without laser irradiation with different exposure parameters, with CUR following wavelengths of 460 nm and MB 660 nm in 15 groups of 10 samples each. After the cultures of the samples the CFU were counted after 24 h of incubation at 37 °C maximum number of colonies was observed in the positive control group (CFU = 201,500 ± 42,093), while the minimum number was detected in the laser group 460 nm + 10.2% CUR (CFU = 10,100 ± 2558), followed by the nystatin group (CFU = 22,300 ± 5578). There was a statistically significant difference between the 460 nm + CUR laser group and the other groups studied ($p < 0.0001$). In conclusion, the 460 nm laser in combination with CUR had the maximum antifungal efficiency against *C. albicans*. The results showed that the MB group and the laser group had no significant differences with the positive control group. In the present study, the type of photosensitizer and laser significantly affected the viability of *C. albicans*, compared to the efficiency of PDT (Daliri et al., 2019).

A study by Zago et al. (2020) reinforced that antimicrobial photodynamic therapy (aPDT) has stood out as one option and promising method of disinfection, having been used for the treatment of oral bacteria. They evaluated *in vitro* the action of aPDT, mediated by MB, chlorin-e6 and curcumin (CUR) against metronidazole-resistant subgingival clinical plaques. The biofilm prepared was a bacterial suspension pool (bacterial plaque was obtained from the periodontal pocket of patients in teeth with bleeding on probing and pocket depth greater than 5 mm) (Fontana et al., 2009). Cell viability in the planktonic and biofilm phase was evaluated by CFU/mL. The aPDT associated with the three photosensitizers tested reduced the total planktonic microbial load and partially reduced the samples in the biofilm. The analysis performed by confocal fluorescence microscopy (CLSM) showed that the photosensitizers used in association with of aPDT permeated the interior of the biofilm. aPDT has been shown to be useful and effective in a complementary approach to the treatment of periodontal disease (Zago et al., 2020).

Li et al. (2021) carried out *in vitro* research to compare the use of dye MB and rose bengal (RB) in antimicrobial photodynamic therapy (PDT) to combat *Enterococcus faecalis* in planktonic and from biofilm potentiated by potassium iodide (KI), a non-toxic inorganic salt. Dentin discs were prepared and placed in contaminated well plates, followed by the addition of a volume of the different photosensitizers and KI and subsequently irradiated. *E. faecalis* in the planktonic form were exposed to antimicrobial PDT with MB and RB activation protocols, with or without KI potentiation. Continuous wave diode lasers were used for irradiation (red light: $\lambda = 660$ nm and green light: $\lambda = 565$ nm), at a laser power of 60 mW/cm² for the different photosensitizers. Potassium iodide (KI) has been shown to exhibit antimicrobial potentiating activities on PDT without adverse

effects and staining problems. Results demonstrated KI helps to avoid staining problems associated with high concentrations of PSs. A combination of antimicrobial KI and PDT can be an effective alternative to frequent methods disinfection in endodontic treatment. More studies are needed with a focus on optimizing laser parameters. The effectiveness of antimicrobial PDT potentiated by KI should be further validated in animal models and clinical studies, however diode laser irradiation in the parameters resulted in a significant reduction of *E. faecalis* bacteria in the form of biofilm. Therefore, PDT activated by MB and RB at low concentrations, or potentiated by KI, can achieve an effect like conventional root canal disinfection (Li et al., 2021).

The limitations are restricted to the few clinical trials found, as well as to some studies that did not mention parameters related to the laser used, such as power and energy density and irradiation time, (Azizi et al., 2019; Freitas et al., 2019; Filipini et al., 2019; Daliri et al., 2019; Li et al., 2021) and regarding photosensitizers, research did not inform the pre-irradiation time (Azizi et al., 2019; Freitas et al., 2019; Javali et al., 2019; Daliri et al., 2019).

In vitro studies, although in a reasonable number, have demonstrated the potential of MB-mediated PDT and their findings represent an evolution in this field. However, only *in vivo* studies can allow the elaboration of an effective protocol. Therefore, *in situ* and *in vivo* investigations are essential to establish a PDT protocol associated with MB that can be safely used in clinical trials.

In this review, a growing, albeit slow, increase was observed in of the application of PDT with MB in clinical trials, in contrast to other studies on the same topic. We emphasize the use of MB with new formulations, demonstrating satisfactory results, potentiating the antimicrobial effect of PDT and minimizing some limitations of the use of MB as a photosensitizer, a topic not addressed in other systematic reviews. It is opportune to reinforce the need for more studies that determine the clinical parameters, establishing specific therapeutic protocols that allow their execution based on science. Although Photodynamic Therapy has shown to be a promising therapy for oral alterations, further studies on the effectiveness of PDT are needed to increase evidence regarding the effectiveness of treatments and to define the parameters (type of light source and power of the light source, exposure time, PS pre-irradiation time) that must be used.

For Wadhwa et al. (2021) the future prospects for PDT are numerous, as techniques and studies have emerged on this advent of modern dentistry.

5. Conclusion

It was evident from the scientific literature that antimicrobial PDT highlights the use of laser and LED, being the most used laser in this therapy. The publications evaluated in the present study described research with PDT using MB, a photosensitizer widely used with reliability, which, even using different protocols from each other, demonstrated the effectiveness of this association of PDT with MB.

This therapy using methylene blue is being accepted as effective in the treatment of various oral curative conditions and has been shown to be a useful and effective therapy as well as a complementary approach to controlling the development/growth of oral biofilm in clinical situations.

The present review demonstrated that the use of antimicrobial PDT mediated by MB can stimulate the development of studies that result in more effective clinical protocols.

The application of PDT in Dentistry has wide indication and already presents satisfactory results, however more studies are needed on the subject to define the parameters such as (type of source and power of the light source, exposure time, photosensitizer) so that consensus is reached on its use in clinical practice.

The effectiveness of photodynamic therapy in the control of oral biofilm using MB among other photosensitizers will be evaluated on an experimental research based on the results obtained in this review.

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