Title

Influence of antimicrobial photodynamic therapy as an adjunctive to scaling and root planing on alveolar bone loss: a systematic review and meta-analysis of animal studies

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Conflict of interest statement: The authors declare no conflict of interest.

Abstract

Background: The study aimed to evaluate the effect of antimicrobial photodynamic therapy (aPDT) as adjunctive therapy to scaling and root planing in experimental periodontitis in rats, with or without systemic involvement, by means of histometric analysis of the furcation region.

Methods: Systematic search was done using PubMed/MEDLINE, SCOPUS, EMBASE and ProQuest databases. Quantitative analysis of alveolar bone loss, with subcategories for the experimental periods studied, was performed. The analysis was performed through the mean difference (MD), with 95% confidence intervals (CIs) and according to SYRCLE guidelines.

Results: Nine studies were considered eligible. A statistically favorable difference was observed for the use of aPDT in all periods studied in systemically healthy animals at 7 ($P < .00001$; MD: −0.71; 95% CI: [−0.85, −0.58]; I2: 90%), 15 ($P < .00001$; MD: −0.49; 95% CI: [−0.62, −0.37]; I2: 88%), and 30 ($P < .00001$; MD: −0.53; 95% CI: [−0.65, −0.41]; I2: 80%) days postoperatively. The difference was also observed for modified animals at 7 ($P < .00001$; MD: −1.03; 95% CI: [−1.43, −0.62]; I2: 97%), 15 ($P < .00001$; MD: −1.04; 95% CI: [−1.62, −0.46]; I2: 99%), and 30 ($P < .00001$; MD: −0.88; 95% CI: [−1.37, −0.39]; I2: 97%) days postoperatively.

Conclusion: The adjunctive use of aPDT favored the reduction of alveolar bone loss in experimental periodontitis in rats, and this result was more evident in systemically compromised rats.

Keywords: periodontal disease, photochemotherapy, alveolar bone loss, systematic review

1. Introduction

Periodontal disease is the most prevalent disease associated with bone loss in adults [1]. The treatment of periodontal disease involves mechanical removal of the buccal biofilm or maintenance of therapeutic concentrations of antimicrobials in the buccal cavity; however, both procedures present limitations [2, 3] and require adjuvant methods to complement the mechanical treatment of scaling and root planing (SRP) [4, 5]. Systemic administration of antibiotics has often been used and appears to promote clinical benefits [6-8]. Nevertheless, microorganisms can rapidly develop resistance against a variety of microbial agents [9], and an effective alternative antimicrobial therapy against Gram-negative bacterial, mycobacterial, fungal, viral, and protozoal pathogens [9] such as antimicrobial photodynamic therapy (aPDT) is required [10, 11].

aPDT may be advantageous as an adjunctive therapy to the conventional treatment of periodontitis [4, 8, 12-19], because of its microbial selectivity, preventing damage to the host tissue around the infected area [10]. In addition to the antimicrobial effects, the use of low-powered lasers seems to have a positive influence on the response of periodontal tissue [10, 11]. The basic principle of aPDT involves the combination of visible or near infrared light, oxygen, and a photosensitizer that is able to absorb and transfer energy or electrons after light absorption to molecular oxygen to generate reactive oxygen species [9].

aPDT has been widely studied as an adjunctive therapy to conventional periodontal treatment [4, 8, 12-24]. *In vitro* studies initially demonstrated benefits (eg, reducing some periodontopathogens) [25-28]. Furthermore, some *in vivo* studies have assessed the effect of aPDT on alveolar bone loss reduction and clastogenic activity, as well as reduction of inflammation in animals [20-24, 29-36]. However, clinical studies in humans have demonstrated variable results [37-41]. Such controversies may be related to the irradiation parameters of the light source, pre-irradiation time, type, and concentration, frequency, and mode of application of the photosensitizer.

Some meta-analyses and systematic reviews have evaluated the adjuvant use of aPDT in the periodontal treatment of patients with chronic periodontitis [37, 38, 41], and aggressive

periodontitis [39], as well as patients with systemic impairment (diabetes) and in the treatment of residual pockets [40]. However, it has not been evaluated the effect of aPDT on the bone tissue of areas with periodontitis in systematic reviews/meta-analysis or clinical studies. Furthermore, the results of experimental animal studies have not been evaluated and discussed in order to compare the parameters of aPDT regarding its real effectiveness in the control of alveolar bone loss. Therefore, the purpose of the present study was to assess the effects of aPDT as an adjunctive therapy to SRP (compared to SRP alone) on alveolar bone loss in experimental periodontitis in rats through a systematic review and meta-analysis.

2. Material and Methods

The review methodology was specified in advance and documented according to the SYRCLE systematic review protocol for animal intervention studies [42].

2.1 Eligibility criteria

The studies selected for this analysis followed the criteria established by the PICO strategy. The PICO question elaborated was "What are the effects of aPDT as an adjunctive therapy to SRP compared to SRP alone on alveolar bone loss in experimental periodontitis in rats?" Criteria considered were (1) population (a model of experimental periodontitis in rats); (2) intervention (rats treated with aPDT as an adjunct to SRP); (3) comparison (rats that received SRP alone followed by irrigation with saline solution); and (4) outcomes (alveolar bone loss in furcation region).

The following eligibility criteria were used: (1) animal studies; (2) studies with a model of experimental periodontitis in Wistar rats induced by a cotton ligature placed in a submarginal position for 7 days; (3) studies that compared conservative SRP to the use of aPDT as an adjunctive therapy to SRP in experimental periodontitis in rats; (4) the presence of a control group (rats receiving SRP without adjunctive aPDT); and (5) studies that assessed the alveolar bone loss in the furcation region by histometric analyses. Exclusion criteria adopted were

review papers, clinical trials, case reports, letters to the editor, commentaries, interviews, updates, in vitro studies, and studies that assessed the use of aPDT as monotherapy.

2.2 Search and information sources

PubMed/MEDLINE, SCOPUS, and EMBASE databases were searched by two authors (M.A.A.N. and D.M.J.M.) independently, according to the inclusion criteria and without any language or publication status restriction. Dissertations and theses were searched using the ProQuest Dissertations and Theses database. A manual complementary search was also conducted in the following journals: *Journal of Periodontology*, *Journal of Clinical Periodontology*, *Lasers in Medical Science*, *Lasers in Surgery and Medicine*, and *Journal of Photochemistry and Photobiology B: Biology*. The search strategies developed for each database are presented in a supplementary file (Supplementary File 1). The search was performed from November 2017 to May 2018. All searches were updated on October 9, 2018.

Screening was performed in two phases. The articles were initially prescreened based on title, followed by analysis of the abstract. Articles considered eligible were subsequently analyzed within inclusion and exclusion criteria through full-text screening. Two blind reviewers (M.A.A.N. and D.M.J.M) independently assessed each article, and discrepancies were resolved by a third reviewer (L.H.T). Search results from each database were combined and duplicates removed.

2.3 Study characteristics and data extraction

The full-text review and data collection were independently performed by two reviewers. After reading the articles, data were collected by one author (M.A.A.N.), and a second author (M.B.O) was responsible for checking all tabulated data. Any discrepancies were solved by mutual discussion, and if unsolved, a third reviewer (L.H.T) was consulted. The information from the accepted studies was tabulated according to the animal species, gender, quantity, and age of animals, as well as by weight, systemic condition, experimental periodontitis, control groups, type of laser used for aPDT, photosensitizer and pre-irradiation time, laser parameters, irradiation method, experimental periods (time points), processing for microscopic analyses, mean of bone loss in millimeters squared, and standard deviation.

2.4 Data items and risk of bias

Risk of bias in the included studies was assessed using SYRCLE's risk of bias tool for animal studies. [43]. The assessment of risk of bias was conducted independently by two reviewers (M.A.A.N and D.M.J.M), and in cases of doubt a third reviewer (L.H.T) participated in order to solve discrepancies.

2.5 Collection of outcome data and data synthesis

Details of the experiment (animal species, gender, number of animals and age, weight, systemic condition, experimental periodontitis induction, experimental groups analyzed, and experimental periods) as well as descriptive parameters used in aPDT therapy (laser types, wavelengths, spot size, power, energy density, total energy, type and concentration of photosensitizer, pre-irradiation time, irradiation time, and laser irradiation method) were extracted for descriptive analysis by two reviewers (M.A.A.N and E.E.). The outcome data of alveolar bone loss was extracted from tables and the corresponding authors were contacted for missing data in the paper. All the included studies assessed alveolar bone loss in the furcation region of molars by histometric analysis. The data were presented by mean bone loss in millimeters squared and standard deviation.

2.6 Meta-analysis

In order to estimate the effect of the treatment with aPDT as an adjunctive therapy to SRP, a quantitative analysis was performed from histometric data of mean and standard deviation of alveolar bone loss in millimeters squared in the furcation region of molars. Effect estimates are reported as mean difference (MD) with the corresponding 95% confidence intervals (CIs). Due to the presence of studies with experimental groups with systemically modified animals, the analyses were conducted separately in two groups: (1) systemically healthy animals and (2) systemically modified animals. Within each of these groups, subgroups were created to analyze the effects of aPDT in each experimental period (7, 15, and 30 days postoperative). Studies that presented more than 1 experimental group of aPDT, with variations in the concentration of the photosensitizer or time and number of laser applications, had more than 1 analysis per article.

Heterogeneity between the studies was evaluated with an I^2 test and data were considered heterogeneous for an I^2 value higher than 40%. The random-effects model was chosen. The analysis was performed using Review Manager (version 5.3.; The Cochrane Collaboration, 2014, Oxford, UK).

3. Results

3.1 Study selection and characteristics

A flow chart of the study selection is represented in Figure 1. The electronic search strategy retrieved a total of 778 records, of which 170 were excluded as duplicate records and 608 were excluded after a detailed screening of titles and abstracts. Nineteen full-text studies were assessed for eligibility. Of these 19 remaining articles, 10 papers were excluded because they met one or more exclusion criterion (Supplemental File 2) and 9 were included in this systematic review and meta-analysis. The meta-analysis was conducted independently among studies with rats that were systemically healthy [20, 21, 29-33, 36] and rats that were systemically modified [29-33, 35, 36].

The overview of characteristics processed and groups included in each article are presented in Table 1. All studies used adult Wistar rats, with a male gender predilection and weight range of 200 g to 300 g. Only three studies [32, 33, 36] were conducted in female adult rats.

Two studies [20, 21] had no groups of systemically modified animals. Considering the methods to induce systemic conditions, studies administered intravenous injection of alloxan (42 mg/kg body weight concentration) for inducing diabetes [29], subcutaneous injection of dexamethasone (2 mg/kg) for immunosuppression induction [30], subcutaneous injection of nicotine hemisulphate preparation (3 mg/kg) [31], surgical procedure for ovariectomy [32], subcutaneous insertion of osmotic minipumps containing nicotine [33], intraperitoneal injections of 5-fluorouracil (50 mg/mL) [35] and surgical procedure for ovariectomy followed by the subcutaneous insertion of osmotic mini-pumps containing nicotine [36].

All experimental treatments were performed with a wavelength of 660 nm from two types of low-powered lasers (GaAlAs and InGaAlP). The photosensitizer toluidine blue O (TBO) at a concentration of 100 μg/mL was the most used, and all the studies employed a pre-irradiation time of 60 seconds. The other characteristics regarding the parameters of the lasers used and the protocols for aPDT are described in Table 2.

3.2 Analysis of bias

Figure 2 and Figure 3 present the classification of bias of the studies included in this systematic review. Information about caregivers and/or investigators blinded from the knowledge of which intervention each animal received during the experiments was unclear in all studies. Two studies did not describe a random component in the sequence generation process [29, 33] and one study did not describe the allocation method to the different treatments groups adequately [29].

3.3 Systemically healthy animals

Statistically favorable differences were found in the use of aPDT in association with SRP for alveolar bone loss reduction for all experimental periods assessed; that is, at 7 ($P <$ .00001; MD: −0.71; 95% CI: [−0.85, −0.58]; I^2: 90%; Figure 4a), at 15 ($P <$ .00001; MD: −0.49; 95% CI: [−0.62, −0.37]; I^2: 88%; Figure 4b), and at 30 ($P <$ .00001; MD: −0.53; 95% CI: [−0.65, −0.41]; I^2: 80%; Figure 4c) days postoperatively.

3.4 Systemically modified animals

The evaluation of the effect of aPDT as an adjunctive therapy in the treatment of experimental periodontitis in systemically modified rats showed statistically favorable differences in the use of aPDT to reduce alveolar bone loss in all experimental periods assessed; that is, at 7 ($P <$ .00001; MD: −1.03; 95% CI: [−1.43, −0.62]; I^2: 97%; Figure 5a), at 15 ($P <$ .00001; MD: −1.04; 95% CI: [−1.62, −0.46; I^2: 99%; Figure 5b), and at 30 ($P <$ .00001; MD: −0.88; 95% CI: [−1.37, −0.39]; I^2: 97%; Figure 5c) days postoperatively.

4. Discussion

This systematic review and meta-analysis selected studies that assessed the effects of aPDT as an adjunctive therapy to SRP compared to SRP alone on alveolar bone loss in experimental periodontitis in rats. The results of the meta-analysis showed that the application of aPDT in association with SRP treatment was favorable to reducing alveolar bone loss in the furcation region in all postoperative periods evaluated for systemically healthy animals [20, 21, 29-33, 36] and systemically modified animals [29-33, 35, 36].

Conventional periodontal treatment results in the repair of inflamed periodontal tissue and the control of alveolar bone loss; however, due to anatomical and instrumentational physical limitations, SRP treatment can fail to remove all subgingival plaque and calculus [44]. Histometric analysis in animal models may contribute to more detailed measurement of the effect of adjunctive periodontal treatments to SRP on bone loss, especially in the furcation region, because minor differences are not clinically detected by depth probing parameters in clinical studies [37].

Different animal models are appropriate for examining the components involved in the interactions between host bacteria and periodontal diseases [45]. Alveolar bone loss in the ligature model is, like human periodontitis, dependent on bacteria [46]. Ligand-induced experimental periodontitis is associated with a host response that involves the accumulation of an inflammatory infiltrate in the gingiva prior to bone resorption [47], and that is sensitive to systemic effects [48, 49]. However, besides the similarities between humans and animals in the formation of periodontal pockets, it is assumed that preclinical models cannot completely reproduce human periodontal pockets/intrabony defects and the extrapolation of the results to humans should be limited [50].

Overall, the aPDT protocols used in the studies demonstrated efficacy in reducing alveolar bone loss in the furcation region [20, 21, 29-33, 35, 36]. The studies included in this review used the phenothiazine photosensitizers methylene blue (MB) and TBO at concentrations of 100μg/mL and 10 mg/mL. Furthermore, one of the studies evaluated the effect of the concentrations of photosensitizers on bone loss [21]. The results demonstrated that a concentration of 100 μg/mL of MB or TBO is the most beneficial in controlling bone loss. The

higher reduction of bone loss at smaller concentrations of the photosensitizers can be explained by the aggregational behavior of photosensitizers. Aggregation is a common phenomenon associated with planar molecules, which usually increase with concentration. Molecules within the body of the aggregate are consequently not reached by incident light and photosensitization is only possible at the surface [51].

The pre-irradiation time of 60 seconds was a consensus among the studies. However, no agreement was observed in the literature regarding the time required for an adequate biodistribution in periodontal pockets. The pre-irradiation time used in clinical studies ranges from 5 to 300 seconds [8, 12, 13, 15-17, 19, 41, 52, 53]. A short incubation period of the photosensitizer prior to illumination favors its binding with microorganism and minimizes penetration into the host tissue, which is observed only after several hours. This provides a distinct and selective therapeutic advantage for photoantimicrobials compared to conventional medicaments due to the rapid uptake of the photosensitizer by target cells relative to the host, combined with physically directed illumination [54].

Additionally, effective photoantimicrobial action is achieved with an efficient light source that has the correct wavelength range and sufficient power output [51]. On a molecular level, photoantimicrobial action involves the absorption of light of a specific wavelength by a photosensitizer to enable the promotion of a paired ground state electron to the singlet excited state [51, 55]. Thus, the wavelength of the light that will be used should be similar to the absorption spectrum of the photosensitizer. MB (max 660 nm in aqueous solution) and TBO (625 nm) exhibit intense light absorption in the red region of the spectrum [56]. All studies included in this review used low-powered lasers with a wavelength of 660 nm.

In addition to the criteria mentioned above, the energy density may range according to the laser emitter used, the output power, irradiation time, and the output diameter of the beam [57]. The analyzed studies showed little variation of these parameters. The laser-emitting sources used were GaAlAs (power of 0.03 W) and InGaAlP (a power of 0.035 W), with a spot size of 0.07 cm^2 and 0.0283 cm^2, respectively. The transgingival irradiation method did not differ among studies either. All aPDT treatments were performed with the laser positioned

perpendicular to the long axis of the tooth and in contact with the gingiva. The application points varied from 3 to 1 points in each buccal and lingual aspect, with the application time ranging from 133 s/point to 12 s/point. These differences in irradiation time were related to the variation of power between lasers and the spot size.

Although no significant differences were found in the treatment protocols among papers, studies conducted on female rats obtained the smallest differences between treatments in most subgroup analyses [32, 33, 36]. Such differences in response to the treatments between male and female rats suggest that the gender of the animal should be considered during the design of experimental studies in animals.

The meta-analysis of systemically modified rats showed a greater mean difference in the reduction of bone loss compared to that observed in systemically healthy rats in all postoperative periods. This can be explained by the compensatory action of aPDT as an adjunctive therapy against intrinsic alterations related to the process of periodontal repair after induced diseases, such as delay in the healing process [58], imbalance in the remodeling sequence of periodontal tissues [59, 60] and damage to the structural integrity of the junctional and sulcular epithelium [61].

The effects of aPDT over the control of alveolar bone loss might be consequence of its bactericidal activity against periodontopathogens, which was previously demonstrated both by *in vitro* and *in vivo* studies [62]. It is also suggested that aPDT modulates inflammatory response through reduction of expression of pro-inflammatory cytokines [63], consequently affecting RANKL/OPG system, that leads to reduction of bone loss [21,33]. Hence, combined with its antimicrobial effects, aPDT can also act over bone repair by accelerating the healing process though low-intensity laser photobiomodulation [29,31].

Despite the positive results in the use of aPDT as an adjunctive therapy to nonsurgical periodontal treatment on the reduction of alveolar bone loss, the present review and meta-analysis have some limitations and the interpretation of results must be carried out carefully. The inclusion and exclusion criteria adopted to guarantee a careful analysis of the primary outcome of bone loss unfortunately restricted the analysis to studies from the same research

group. Although the selected studies for the present meta-analysis used very similar treatment protocols and identical experimental times, a high heterogeneity in the results was observed. It is believed that this heterogeneity may be explained by the variation of animal weight and gender. Meta-analyses of animal studies usually show greater heterogeneity due to the exploratory nature of animal studies compared to clinical research and an unavoidable heterogeneity between animal studies [64]. Nevertheless, the high heterogeneity in the analysis of the subgroups of systemically modified animals can be explained by the variety of disorders studied, which results in immune-inflammatory responses with different proportions.

5. Conclusion

The adjunctive use of aPDT, independent of phenothiazinium photosensitizer and protocols of low-power laser used, favored the reduction of alveolar bone loss in experimental periodontitis in rats, and this result was more evident in systemically compromised rats.

Acknowledgement

Role of funding source: This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

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Figure Legends

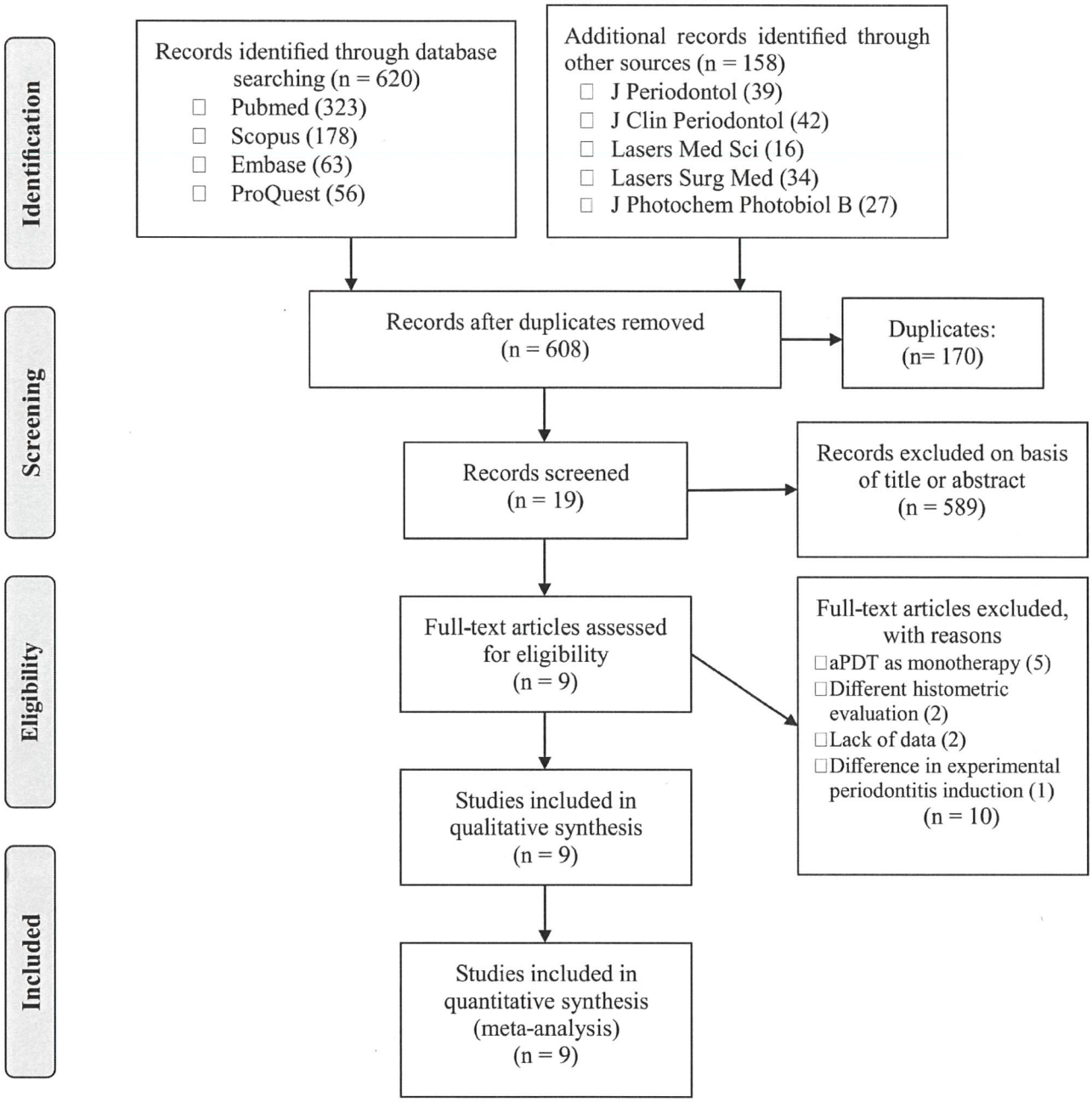
Figure 1. Flow diagram of the search strategy.

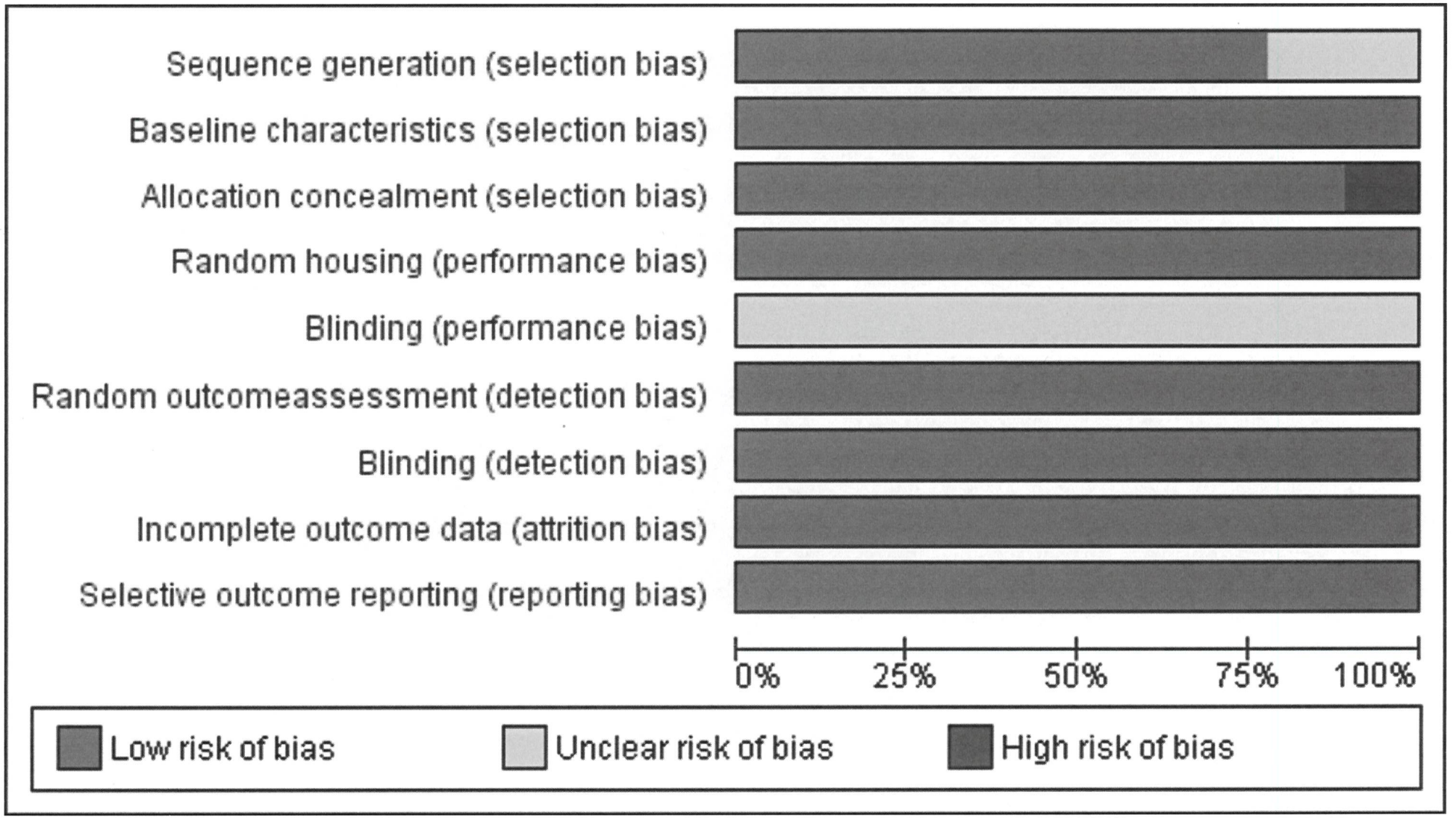
Figure 2. Risk of bias in included studies by SYRCLE's risk of bias tool for animal studies.

Figure 3. Summary of the risk of bias assessment according to the SYRCLE's risk of bias tool for animal studies.

Figure 4. Forest plot comparison between aPDT as an adjunctive therapy to SRP and SRP alone on alveolar bone loss in experimental periodontitis in rats systemically healthy at 7 (a), 15 (b) and 30 (c) days.

Figure 5. Forest plot comparison between aPDT as an adjunctive therapy to SRP and SRP alone on alveolar bone loss in experimental periodontitis in rats systemically modified at 7 (a), 15 (b) and 30 (c) days.


Identification
Screening
Eligibility
Included
Records identified through database searching (n = 620)
Pubmed (323)
Scopus (178)
Embase (63)
ProQuest (56)
Additional records identified through other sources (n = 158)
J Periodontol (39)
J Clin Periodontol (42)
Lasers Med Sci (16)
Lasers Surg Med (34)
J Photochem Photobiol B (27)
Records after duplicates removed (n = 608)
Duplicates: (n= 170)
Records screened (n = 19)
Records excluded on basis of title or abstract (n = 589)
Full-text articles assessed for eligibility (n = 9)
Full-text articles excluded, with reasons
aPDT as monotherapy (5)
Different histometric evaluation (2)
Lack of data (2)
Difference in experimental periodontitis induction (1)
(n = 10)
Studies included in qualitative synthesis (n = 9)
Studies included in quantitative synthesis (meta-analysis) (n = 9)

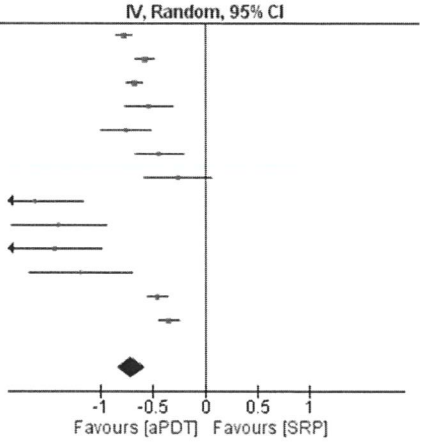

Sequence generation (selection bias)
Baseline characteristics (selection bias)
Allocation concealment (selection bias)
Random housing (performance bias)
Blinding (performance bias)
Random outcomeassessment (detection bias)
Blinding (detection bias)
Incomplete outcome data (attrition bias)
Selective outcome reporting (reporting bias)
0%
25%
50%
75%
100%
Low risk of bias
Unclear risk of bias
High risk of bias

Sequence generation (selection bias)
Baseline characteristics (selection bias)
Allocation concealment (selection bias)
Random housing (performance bias)
Blinding (performance bias)
Random outcomeassessment (detection bias)
Blinding (detection bias)
Incomplete outcome data (attrition bias)
Selective outcome reporting (reporting bias)
Almeida et al., 2008
Fernandes et al., 2009
Garcia et al, 2011
Garcia et al., 2013
Garcia et al., 2013b
Garcia et al., 2014
Garcia et al., 2018
Gualberto et al., 2016
Theodoro et al. 2017

a)

Study or Subgroup	aPDT Mean	aPDT SD	aPDT Total	SRP Mean	SRP SD	SRP Total	Weight	Mean Difference IV, Random, 95% CI
Almeida et al.,2008	0.33	0.05	10	1.11	0.11	10	10.3%	-0.78 [-0.85, -0.71]
Fernandes et al., 2009	0.54	0.06	10	1.12	0.13	10	10.1%	-0.58 [-0.67, -0.49]
Garcia et al, 2011	0.41	0.03	10	1.09	0.13	10	10.2%	-0.68 [-0.76, -0.60]
Garcia et al., 2013a (aPDT1)	0.82	0.1	5	1.36	0.24	5	8.1%	-0.54 [-0.77, -0.31]
Garcia et al., 2013a (aPDT2)	0.6	0.12	5	1.36	0.24	5	8.0%	-0.76 [-1.00, -0.52]
Garcia et al., 2013a (aPDT3)	0.92	0.1	5	1.36	0.24	5	8.1%	-0.44 [-0.67, -0.21]
Garcia et al., 2013b	0.84	0.44	10	1.1	0.28	10	6.5%	-0.26 [-0.58, 0.06]
Garcia et al., 2014 (G6)	0.55	0.14	6	2.17	0.54	6	4.9%	-1.62 [-2.07, -1.17]
Garcia et al., 2014 (G7)	0.77	0.17	6	2.17	0.54	6	4.8%	-1.40 [-1.85, -0.95]
Garcia et al., 2014 (G8)	0.73	0.17	6	2.17	0.54	6	4.8%	-1.44 [-1.89, -0.99]
Garcia et al., 2014 (G9)	0.98	0.3	6	2.17	0.54	6	4.3%	-1.19 [-1.68, -0.70]
Garcia et al., 2018	0.88	0.15	10	1.34	0.07	10	10.0%	-0.46 [-0.56, -0.36]
Gualberto et al., 2016	0.71	0.12	10	1.06	0.11	10	10.0%	-0.35 [-0.45, -0.25]
Total (95% CI)			**99**			**99**	**100.0%**	**-0.71 [-0.85, -0.58]**

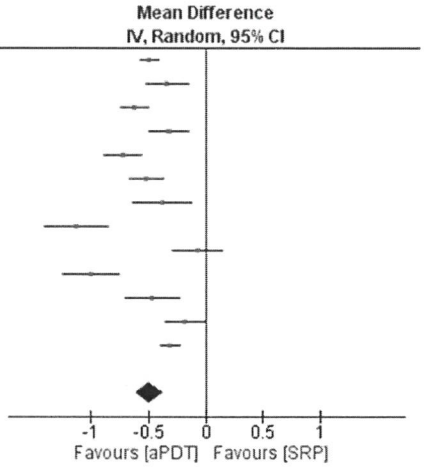
Heterogeneity: $Tau^2 = 0.04$; $Chi^2 = 114.55$, df = 12 ($P < 0.00001$); $I^2 = 90\%$
Test for overall effect: $Z = 10.43$ ($P < 0.00001$)



b)

Study or Subgroup	aPDT Mean	aPDT SD	aPDT Total	SRP Mean	SRP SD	SRP Total	Weight	Mean Difference IV, Random, 95% CI
Almeida et al.,2008	0.35	0.06	10	0.845	0.12	10	9.0%	-0.49 [-0.58, -0.41]
Fernandes et al., 2009	0.56	0.13	10	0.9	0.27	10	7.7%	-0.34 [-0.53, -0.15]
Garcia et al, 2011	0.4	0.02	10	1.02	0.19	10	8.6%	-0.62 [-0.74, -0.50]
Garcia et al., 2013a (aPDT1)	1.02	0.13	5	1.34	0.15	5	7.9%	-0.32 [-0.49, -0.15]
Garcia et al., 2013a (aPDT2)	0.62	0.1	5	1.34	0.15	5	8.1%	-0.72 [-0.88, -0.56]
Garcia et al., 2013a (aPDT3)	0.82	0.08	5	1.34	0.15	5	8.2%	-0.52 [-0.67, -0.37]
Garcia et al., 2013b	0.6	0.09	10	0.98	0.41	10	6.6%	-0.38 [-0.64, -0.12]
Garcia et al., 2014 (G6)	0.54	0.21	6	1.66	0.28	6	6.3%	-1.12 [-1.40, -0.84]
Garcia et al., 2014 (G7)	1.59	0.02	6	1.66	0.28	6	7.1%	-0.07 [-0.29, 0.15]
Garcia et al., 2014 (G8)	0.66	0.11	6	1.66	0.28	6	6.9%	-1.00 [-1.24, -0.76]
Garcia et al., 2014 (G9)	1.19	0.1	6	1.66	0.28	6	6.9%	-0.47 [-0.71, -0.23]
Garcia et al., 2018	0.84	0.15	10	1.02	0.23	10	7.9%	-0.18 [-0.35, -0.01]
Gualberto et al., 2016	0.68	0.12	10	0.99	0.08	10	8.9%	-0.31 [-0.40, -0.22]
Total (95% CI)			**99**			**99**	**100.0%**	**-0.49 [-0.62, -0.37]**

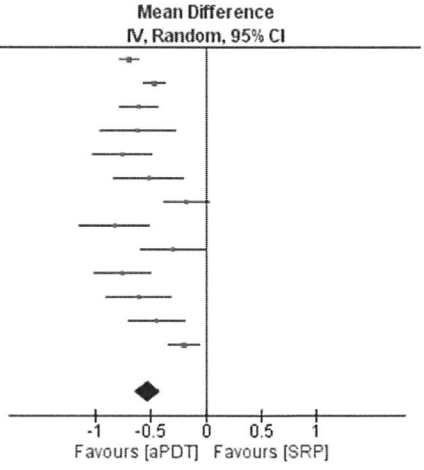
Heterogeneity: $Tau^2 = 0.04$; $Chi^2 = 96.62$, df = 12 ($P < 0.00001$); $I^2 = 88\%$
Test for overall effect: $Z = 7.87$ ($P < 0.00001$)



c)

Study or Subgroup	aPDT Mean	aPDT SD	aPDT Total	SRP Mean	SRP SD	SRP Total	Weight	Mean Difference IV, Random, 95% CI
Almeida et al.,2008	0.27	0.07	10	0.97	0.13	10	10.2%	-0.70 [-0.79, -0.61]
Fernandes et al., 2009	0.53	0.05	10	1	0.16	10	10.0%	-0.47 [-0.57, -0.37]
Garcia et al, 2011	0.38	0.05	10	0.99	0.28	10	8.8%	-0.61 [-0.79, -0.43]
Garcia et al., 2013a (aPDT1)	0.72	0.27	5	1.34	0.28	5	5.9%	-0.62 [-0.96, -0.28]
Garcia et al., 2013a (aPDT2)	0.58	0.11	5	1.34	0.28	5	7.2%	-0.76 [-1.02, -0.50]
Garcia et al., 2013a (aPDT3)	0.82	0.23	5	1.34	0.28	5	6.2%	-0.52 [-0.84, -0.20]
Garcia et al., 2013b	0.45	0.26	10	0.63	0.21	10	8.2%	-0.18 [-0.39, 0.03]
Garcia et al., 2014 (G6)	0.55	0.26	6	1.38	0.3	6	6.2%	-0.83 [-1.15, -0.51]
Garcia et al., 2014 (G7)	1.08	0.22	6	1.38	0.3	6	6.6%	-0.30 [-0.60, -0.00]
Garcia et al., 2014 (G8)	0.62	0.1	6	1.38	0.3	6	7.4%	-0.76 [-1.01, -0.51]
Garcia et al., 2014 (G9)	0.77	0.21	6	1.38	0.3	6	6.7%	-0.61 [-0.90, -0.32]
Garcia et al., 2018	0.62	0.11	10	1.07	0.4	10	7.3%	-0.45 [-0.71, -0.19]
Gualberto et al., 2016	0.65	0.06	10	0.85	0.22	10	9.4%	-0.20 [-0.34, -0.06]
Total (95% CI)			**99**			**99**	**100.0%**	**-0.53 [-0.65, -0.41]**

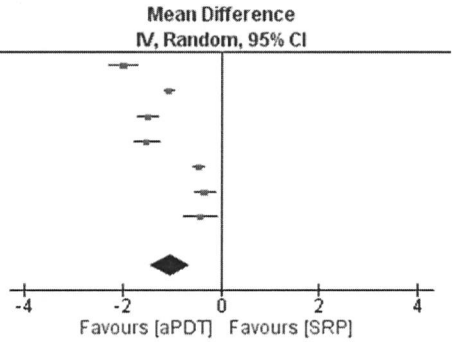
Heterogeneity: $Tau^2 = 0.04$; $Chi^2 = 59.98$, df = 12 ($P < 0.00001$); $I^2 = 80\%$
Test for overall effect: $Z = 8.49$ ($P < 0.00001$)



a)

Study or Subgroup	Experimental Mean	SD	Total	Control Mean	SD	Total	Weight	Mean Difference IV, Random, 95% CI
Almeida et al.,2008	0.29	0.03	10	2.27	0.47	10	13.9%	-1.98 [-2.27, -1.69]
Fernandes et al., 2009	0.6	0.1	10	1.65	0.15	10	14.8%	-1.05 [-1.16, -0.94]
Garcia et al, 2011	0.44	0.08	10	1.92	0.34	10	14.4%	-1.48 [-1.70, -1.26]
Garcia et al., 2013b	0.96	0.26	10	2.46	0.35	10	14.1%	-1.50 [-1.77, -1.23]
Garcia et al., 2018	1.05	0.15	10	1.5	0.12	10	14.8%	-0.45 [-0.57, -0.33]
Gualberto et al., 2016	0.94	0.19	10	1.27	0.27	10	14.5%	-0.33 [-0.53, -0.13]
Theodoro et al. 2017	0.56	0.28	10	0.98	0.49	10	13.5%	-0.42 [-0.77, -0.07]
Total (95% CI)			**70**			**70**	**100.0%**	**-1.03 [-1.43, -0.62]**

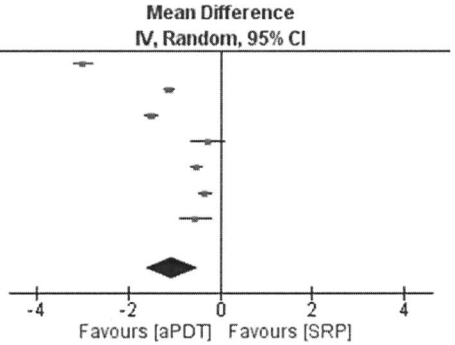
Heterogeneity: Tau² = 0.29; Chi² = 197.67, df = 6 (P < 0.00001); I² = 97%
Test for overall effect: Z = 4.94 (P < 0.00001)



b)

Study or Subgroup	Experimental Mean	SD	Total	Control Mean	SD	Total	Weight	Mean Difference IV, Random, 95% CI
Almeida et al.,2008	0.24	0.02	10	3.23	0.34	10	14.3%	-2.99 [-3.20, -2.78]
Fernandes et al., 2009	0.59	0.13	10	1.71	0.11	10	14.5%	-1.12 [-1.23, -1.01]
Garcia et al, 2011	0.42	0.06	10	1.91	0.21	10	14.5%	-1.49 [-1.63, -1.35]
Garcia et al., 2013b	0.95	0.36	10	1.22	0.44	10	13.9%	-0.27 [-0.62, 0.08]
Garcia et al., 2018	1.1	0.1	10	1.62	0.16	10	14.5%	-0.52 [-0.64, -0.40]
Gualberto et al., 2016	1.26	0.19	10	1.59	0.11	10	14.5%	-0.33 [-0.47, -0.19]
Theodoro et al. 2017	0.67	0.17	10	1.21	0.54	10	13.9%	-0.54 [-0.89, -0.19]
Total (95% CI)			**70**			**70**	**100.0%**	**-1.04 [-1.62, -0.46]**

Heterogeneity: Tau² = 0.61; Chi² = 577.61, df = 6 (P < 0.00001); I² = 99%
Test for overall effect: Z = 3.51 (P = 0.0005)



c)

Study or Subgroup	Experimental Mean	SD	Total	Control Mean	SD	Total	Weight	Mean Difference IV, Random, 95% CI
Almeida et al.,2008	0.27	0.11	10	2.82	0.75	10	13.3%	-2.55 [-3.02, -2.08]
Fernandes et al., 2009	0.57	0.1	10	1.5	0.25	10	14.9%	-0.93 [-1.10, -0.76]
Garcia et al, 2011	0.4	0.03	10	1.89	0.22	10	15.0%	-1.49 [-1.63, -1.35]
Garcia et al., 2013b	0.84	0.45	10	1.24	0.66	10	13.1%	-0.40 [-0.90, 0.10]
Garcia et al., 2018	0.88	0.22	10	1.26	0.09	10	15.0%	-0.38 [-0.53, -0.23]
Gualberto et al., 2016	0.65	0.33	10	1.08	0.24	10	14.6%	-0.43 [-0.68, -0.18]
Theodoro et al. 2017	1.01	0.42	10	1.04	0.42	10	14.0%	-0.03 [-0.40, 0.34]
Total (95% CI)			**70**			**70**	**100.0%**	**-0.88 [-1.37, -0.39]**

Heterogeneity: Tau² = 0.41; Chi² = 204.61, df = 6 (P < 0.00001); I² = 97%
Test for overall effect: Z = 3.53 (P = 0.0004)

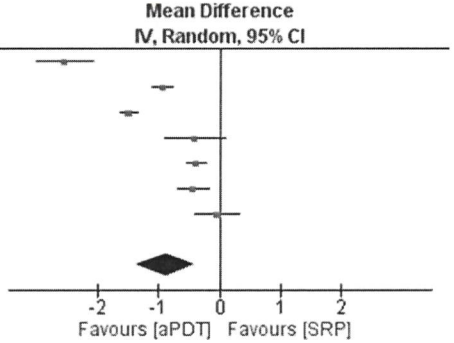


Table 1. Overview of the included studies and characteristics processed for data extraction.

Study ID	Species/ Gender	Number of animals/age	Weight (g)	Systemic condition	Collected treatment groups	aPDT effect
Almeida et al., 2008	Wistar/ Male	240 adult (30/group)	120-140	1.Healthy (ND) 2. Diabetic rats/alloxan (D)	**SRP (in ND and D group)**, SRP and saline solution **aPDT (in ND and D group)**, SRP followed aPDT	Positive
Fernandes et al., 2009	Wistar/ Male	180 adult (30/group)	120-140	1.Healthy (ND) 2. Immunosuppressed rats/ dexamethasone (D)	**SRP (in ND and D group)**, SRP and saline solution **aPDT (in ND and D group),** SRP followed aPDT	Positive
Garcia et al., 2011	Wistar/ Male	240 adult (30/group)	120-140	1.Healthy (C) 2. Nicotine-modified rats (N)	**SRP (in C and N group**), SRP and saline solution **aPDT (in C and N group)**, SRP followed aPDT	Positive
Garcia et al., 2013 (a)	Wistar/ Male	75 adult (15/group)	250-300	Healthy rats	**SRP group**, SRP and irrigation with saline solution; **aPDT**, SRP followed aPDT at 0h* **aPDT2**, SRP followed aPDT at 0, 24, 48 and 72h* **aPDT3**, SRP followed aPDT at 0, 48, 96 and 144h*	Positive
Garcia et al., 2013 (b)	Wistar/ Female	270 adult (30/group)	200-300	1.Healthy (N) 2. Rats with ovariectomy (O)	**SRP (in N and O group)**, SRP and saline solution; **aPDT (in N and O group)**, SRP followed aPDT	Positive
Garcia et al., 2014	Wistar/ Male	162 adult (18/group)	200-300	Healthy rats	**SRP (G1)**, SRP and irrigation with saline solution; **aPDT (G6)**, SRP followed aPDT (MB 100 μg/mL) **aPDT (G7)**, SRP followed aPDT (MB 10mg/mL)	Positive

					aPDT (G8), SRP followed aPDT (TBO 100 µg/mL) **aPDT (G9)**, SRP followed aPDT (TBO 10 mg/mL)	
Gualberto et al., 2016	Wistar/ Female	180 adult (30/group)	266±6.5	1. Healthy (Veh) 2. Nicotine-modified rats (Nic)	**SRP (in Veh and Nic group)**, SRP and saline solution **aPDT (in Veh and Nic group)**, SRP followed aPDT	Positive
Theodoro et al., 2017	Wistar/ Male	150 adult (30/group)	200-300	Immunosuppressed rats with chemotherapy by 5-FU	**5FU/SRP group**, SRP and saline solution **5FU/SRP/aPDT group**, SRP followed aPDT	Positive
Garcia et al., 2018	Wistar/ Female	180 adult (30/group)	266±6.5	1.Healthy (Veh) 2. Rats with ovariectomy under systemic nicotine (Nic)	**SRP group (Veh and Nic)**, SRP and saline solution. **aPDT group (Veh and Nic)**, SRP followed aPDT	Positive

SRP scaling and root planing; *aPDT* antimicrobial photodynamic therapy
*Time of laser postoperative application after the ligature removed

Table 2. Overview of the selected studies and laser parameters of interest.

Study ID	Laser (λ)	Spot size	LPL Parameters	PS Pre-irradiation/ Irradiation time	LPL irradiation method
Almeida et al., 2008	GaAlAs (660nm)	0.07cm²	Power of 0.03W energy of 4 J/point (57.14 J/cm²/point) total energy of 24 J	TBO (100μg/mL) 60s /133s/point	Transgingival. Three equidistant points at each buccal and lingual aspect in contact with the tissue
Fernandes et al., 2009	GaAlAs (660nm)	0.07cm²	Power of 0.03W energy of 4 J/point (57.14 J/cm²/point) total energy of 24J	TBO (100μg/mL) 60s/133s/point	Transgingival. Three equidistant points at each buccal and lingual aspect in contact with the tissue
Garcia et al., 2011	GaAlAs (660nm)	0.07cm²	Power of 0.03W energy of 4 J/point (57.14 J/cm²/point) total energy of 24J	TBO (100μg/mL) 60s/133s/point	Transgingival. Three equidistant points at each buccal and lingual aspect in contact with the tissue
Garcia et al., 2013 (a)	InGaAlP (660nm)	0.0283cm²	Power of 0.035 W energy density of 4.94 J/cm²/point total energy density 29.64 J/cm²	TBO (100μg/mL) 60s /12s/point	Transgingival. Three equidistant points at each buccal and lingual aspect in contact with the tissue
Garcia et al., 2013 (b)	InGaAlP (660nm)	0.0283 cm²	Power of 0.035 W energy density of 4.94 J/cm²/point total energy density of 29.64 J/cm²	TBO (100μg/mL) 60s/4s/point	Transgingival. Three equidistant points at each buccal and lingual aspect perpendicularly and in contact with the gingiva

Garcia et al., 2014	InGaAlP (660nm)	0.0283 cm^2	Power of 0.035 W total energy density of 29.64 J/cm^2	TBO (100µg/mL and 10mg/mL) MB (100µg/mL and 10mg/mL) 60s/12s/point	Transgingival. One point each buccal and lingual aspect perpendicular to the tooth's long axis and in contact with the gingiva
Gualberto et al., 2016	InGaAlP (660nm)	0.0283 cm^2	Power of 0.035 W energy density of 14.82 J/cm^2/point total energy density of 29.64 J/cm^2	TBO (100µg/mL) 60s/12s/point	Transgingival. One point each buccal and lingual aspect of the left mandibular first molar perpendicularly and in contact with the gingivae
Theodoro et al., 2017	InGaAlP (660nm)	0.0283 cm^2	Power of 0.035 W energy density of 14.82 J/cm^2/point total energy density of 29.4 J/cm^2	MB (100µg/mL) 60s /12s/point	Transgingival. One point each buccal and lingual aspect perpendicularly and almost in contact with the gingival tissue
Garcia et al., 2018	InGaAlP (660nm)	0.0283 cm^2	Power of 0.035W energy density of 14.82 J/cm^2/point total energy of 29.64 J/cm^2	TBO (100µg/mL) 60s/12s/point	Transgingival. One point each buccal and lingual aspect perpendicularly and almost in contact with the gingival tissue

GaAlAs gallium-aluminum-arsenide; *InGaAlP* índium gallium aluminum phopohorus; λ wavelength; *LPL* low-power laser; *PS* photosensitizer; *TBO* toluidine blue O; *MB* methylene blue

Supplemental File 1. Search strategies according to electronic source

Source	***Search Strategy***
PUBMED/ MEDLINE	((((("periodontitis"[All Fields] OR "periodontal disease"[All Fields]) OR "periodontal disease/experimental"[All Fields]) OR "periodontitis experimental model"[All Fields]) OR "periodontal diseases"[All Fields]) AND ((("photochemotherapy"[All Fields] OR "photodynamic"[All Fields]) OR ("photodynamic antimicrobial chemotherapy"[All Fields] OR "photodynamic antimicrobial chemotherapy pact"[All Fields])) OR "photodynamic therapy"[All Fields])
EMBASE	(((periodontitis OR 'periodontal disease') AND photochemotherapy OR 'photodynamic therapy' OR 'photodynamic antimicrobial chemotherapy') AND 'scaling and root planing' OR 'periodontal therapy' OR 'root planing') AND ('animal experiment'/exp OR 'animal experiment')
SCOPUS	ALL ("periodontitis") OR ALL ("periodontal disease") OR ALL ("periodontal diseases") AND ALL ("photochemotherapy") OR ALL ("photodynamic therapy") OR ALL ("photodynamic antimicrobial chemotherapy") OR ALL ("photodynamic") AND ALL ("scaling and root planing") OR ALL ("periodontal therapy") OR ALL ("root planing") AND ALL ("animal experiment") OR ALL ("animal model")

Supplemental File 1. Search strategies according to electronic source *(continued)*

Source	***Search Strategy***
ProQuest	periodontitis AND photochemotherapy
J Periodontol J Clin Periodontol Lasers Surg Med	periodontitis OR "periodontal disease" OR "periodontal diseases" AND photochemotherapy OR "photodynamic antimicrobial chemotherapy" OR "photodynamic therapy"
Lasers Med Sci	'periodontitis OR "periodontal disease" OR "periodontal diseases" AND photochemotherapy OR "photodynamic antimicrobial chemotherapy" OR "photodynamic therapy" AND "animal experiment" OR "animal model"'
J Photochem Photobiol B	periodontitis OR "periodontal disease" AND "photodynamic antimicrobial chemotherapy" OR "antimicrobial photodynamic therapy" AND "scaling and root planing" OR "root planing" AND "animal experiment"

Supplemental file 2. Excluded studies and reasons for exclusion.

Publication	***Reasons for exclusion***
J.M. de Almeida, L.H. Theodoro, A.F. Bosco, M.J. Nagata, M. Oshiiwa, V.G. Garcia. Influence of photodynamic therapy on the development of ligature-induced periodontitis in rats. J Periodontol. 78(3) (2007) 566-75.	1.aPDT as monotherapy 2. the ligatures were not removed after 7 days 3. absence of histometric analysis
J.M. de Almeida, L.H. Theodoro, A.F. Bosco, M.J. Nagata, M. Oshiiwa, V.G. Garcia. In vivo effect of photodynamic therapy on periodontal bone loss in dental furcations. J Periodontol. 79(6) (2008) 1081-8.	1.aPDT as monotherapy 2. the ligatures were not removed after 7 days
P.E. Bottura, J Milanezi, L.A. Fernandes, H.C. Caldas, M Abbud-Filho, V.G. Garcia, M.A. Baptista. Nonsurgical periodontal therapy combined with laser and photodynamic therapies for periodontal disease in immunosuppressed rats. Transplant Proc. 43(5) (2011) 2009-16.	1. incomplete data about histometric analyses
A.S. Carvalho, M.H. Napimoga, J. Coelho-Campos, V.J. Silva-Filho, G. Thedei. Photodynamic therapy reduces bone resorption and decreases inflammatory response in an experimental rat periodontal disease model. Photomed Laser Surg. 29 (11) (2011)735-40.	1. incomplete data about histometric analyses
RA Prates, AM Yamada, LC Suzuki, CM França, S Cai, MP Mayer, AC Ribeiro, MS Ribeiro. Histomorphometric and microbiological assessment of photodynamic therapy as an adjuvant treatment for periodontitis: a short-term evaluation of inflammatory periodontal conditions and bacterial reduction in	1. crest bone evaluation

a rat model. Photomed Laser Surg. 29 (12) (2011) 835-44.

Supplemental File 2. Excluded studies and reasons for exclusion *(continued).*

Publication	***Reasons for exclusion***
P.G. de Oliveira, A.M. Silveira E Souza, A.B. Novaes Jr, M. Taba Jr, M.R. Messora, D.B. Palioto, M.F. Grisi, A.C. Tedesco, S.L. de Souza. Adjunctive effect of antimicrobial photodynamic therapy in induced periodontal disease. Animal study with histomorphometrical, immunohistochemical, and cytokine evaluation. Lasers Med Sci.31(7) (2016) 1275-83.	1. 14 days of experimental periodontitis induction
M. de Moraes, R.C. Vasconcelos, J.P. Longo, L.A. Muehlmann, R.B. de Azevedo, R.F. de Araújo Júnior, A.A. Araujo, A. de Lisboa Lopes Costa. Photodynamic therapy using chloro-aluminum phthalocyanine decreases inflammatory response in an experimental rat periodontal disease model. J Photochem Photobiol B. 167 (2017) 208-215.	1. aPDT as monotherapy 2. absence of histometric analysis
Belinello-Souza EL, Alvarenga LH, Lima-Leal C, Almeida P, Leite CG, Lima TR, Godoy-Miranda B, Previati-Oliveira J, de Pretto L, de Freitas AZ, Fernandes AU, Labat Marcos R, Prates RA. Antimicrobial photodynamic therapy combined to periodontal treatment: Experimental model. Photodiagnosis Photodyn Ther. 18 (2017) 275-278.	1. absence of SRP control group; 2. crest bone evaluation.
Camacho-Alonso F, Davia-Peña RS, Vilaplana-Vivo C, Tudela-Mulero MR, Merino JJ, Martínez-Beneyto Y. Synergistic effect of photodynamic therapy and alendronate on alveolar bone loss in rats with ligature-induced periodontitis. J Periodontal Res. (2017) doi: 10.1111/jre.12515.	1. aPDT as monotherapy; 2. ligatures were not removed after 7 days; 3. absence of histometric analysis

Supplemental File 2. Excluded studies and reasons for exclusion *(continued).*

Publication	***Reasons for exclusion***
Theodoro LH, Ferro-Alves ML, Longo M, Nuernberg MAA, Ferreira RP, Andreati A, Ervolino E, Duque C, Garcia VG. Curcumin photodynamic effect in the treatment of the induced periodontitis in rats. Lasers Med Sci. 32(8) (2017) 1783-1791.doi: 10.1007/s10103-017-2261-3.	1. aPDT as monotherapy