# Additional clinical benefits of probiotics as an adjunctive therapy to nonsurgical periodontal treatment of periodontitis: a systematic review and meta-analysis

**Huiqing Gou**
Jiangsu Province Stomatological Hospital

**Xu Chen**
Jiangsu Province Stomatological Hospital

**Lu Li**
Jiangsu Province Stomatological Hospital

**Xiaoqian Wang**
Jiangsu Province Stomatological Hospital

**Yan Xu** ( ✉ yanxu_group@163.com )
Jiangsu Province Stomatological Hospital    https://orcid.org/0000-0002-5496-402X

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**Research article**

# Abstract

Background: With more and more concerns concentrated on the novel therapy applying probiotics, we challenge its trustworthy clinical efficacy as an adjuvant to scaling and root planning (SRP) as compared with SRP alone or combined with placebo applied as the initial therapy of periodontitis.

Methods: Electronic databases retrieval, a grey literature and a hand search were performed until February 2020 under certain screening condition. Clinical randomized controlled trials (RCTs) to assess the efficacy of SRP + probiotic versus SRP treating systemically healthy and nonsmoker individuals diagnosed with periodontitis were included. Primary outcome variables were PPD (pocket probing depth) reduction, CAL (clinical attachment level) gain and the percentage of BOP (bleeding on probing) reduction.

Results: After screening, eleven publications were eligible for the systematic review and ten were evaluated in the meta-analysis. Results demonstrated statistically significant more overall PPD reduction at 1 month (0.48mm, p=0.005), overall CAL gain at 1 (0.35mm, p=0.004) and 3 months (0.13mm, p=0.04) and BOP percentage reduction (10.38, p=0.001) at short-term and 6 months (7.57, p< 0.00001) favoring SRP + probiotics treatment. Moreover, significant more reduction of PPD for moderate (0.19mm, p< 0.00001) and deep pockets (0.58mm, p< 0.00001) and gain of CAL for moderate pockets (0.20mm, p=0.0001) were observed at 3 months favoring adjunctive efficacy of probiotics. However, there were not a significant difference of overall PPD reduction at 3 (0.14mm, p=0.07) and 6 months (0.2mm, p=0.26) and overall CAL gain at 6 months (0.19mm, p=0.53) between two groups.

Conclusions: Within the ranges of this study, the adjunctive use of probiotics seem to achieve short-term clinical benefits in the treatment of periodontitis. Conclusions must be treated with caution because of high heterogeneity among included studies and future long-term RCTs are needed to testify the clinical application value of probiotics.

# Background

Periodontitis is the irreversible chronic inflammatory disease with the initial etiologic factor-plaque biofilm destroying the periodontal support tissue and if untreated, leading to teeth loss, which makes it the unresolved challenge of the oral medicine [1]. It has been profound elucidated that one of the pathologic characteristics of periodontitis is the dysfunctional host response to the oral biofilm attributing to the fluctuation of both the amounts and types of bacteria [2, 3]. It has been universally acknowledged that nonsurgical periodontal therapy is the gold-standard for the management of periodontitis which mainly consists of oral hygiene instructions and scaling and root planning (SRP) [4]. The target of this conventional treatment is to remove adherent and unattached bacterial biofilms as well as deposits of calculus thus reducing inflammation and pocket depths, and promoting periodontal reattachment [5-7]. Unfortunately, such therapeutic approaches are frequently observed with the outcomes of recolonization of treated sites by periodontopathogens, causing the recurrence of the periodontitis [8]. To increase the efficacy of nonsurgical periodontal treatment, adjunctive treatments have been applied such as the use of chlorhexidine, antibiotics, antiseptics or photodynamic therapy to achieve a better decontamination of the periodontal environment [9-11]. Although these nonsurgical treatments have been confirmed to have some certain clinical effectiveness, efforts to improve periodontal therapies through complementary treatments are at research. Recently, probiotic therapy has gained increasing interests among the medical researchers.

Probiotics are living microorganisms, principally bacteria which, when administered in adequate amounts, confer a health benefit to the host [12]. Clinical advances have already been made in the prevention and treatment of systemic gastrointestinal diseases in which polymicrobial intestinal transplants have shown its potential in restoring a balance between the intestine and its microbial inhabitants [13, 14]. Probiotics can exert clinical benefits on the host with the promise to improve and maintain the symbiosis of polymicrobial communities through prevention of adhesion of pathogenic species, inhibition of bacterial growth, modulation of the mucosal immune system or the cell proliferation and improvement of intestinal barrier integrity [15]. Probiotics have been employed as effective adjuncts to control periodontal inflammation [16-19]. The therapeutic effects on the periodontitis have been highlighted in vivo and vitro models [20-22]. However, this is a relatively new field, and data regarding the probiotics therapy in treating periodontitis is scarce.

The objective of this meta-analysis is to analyze the available scientific evidence answering the following focused question developed in accordance with the recognized Patient, Intervention, Comparison and Outcome (PICO) format: 'What is the clinical efficacy of probiotic as an adjunctive therapy of SRP, in terms of PPD reduction, CAL gain and percentage of BOP reduction, when compared with SRP alone or in combination with placebo in the management of periodontitis in humans?'

# Methods

## Protocol

This meta-analysis was prepared in accordance with the PRISMA guidelines [23], and the protocol was registered in the International Prospective Register of Systematic Reviews (PROSPERO, registration number CRD42017083840).

## Search strategy

The following databases: MEDLINE, Cochrane Central Register of Controlled Trials, Science Direct databases, PubMed, Embase, Web of science, Scopus, a grey literature and a hand search were performed from January 2010 to February 2020. The following strategy in the search using Boolean operators and an asterisk symbol (*) as truncation was employed to identify papers using MesH, keywords and other free terms: (Periodontitis OR Chronic periodontitis OR Aggressive periodontitis OR Periodontal disease OR Periodont* OR probing pocket depth OR periodontal pocket) AND (Intervention OR Therapy OR Treatment) OR (Scaling and root planning OR SRP OR nonsurgical periodontal therapy OR non-surgical therapy OR Periodontal treatment OR Periodontal therapy) AND (Probiotic OR Probiotic* OR Probiotic therapy OR Probiotic effect OR Probiotic treatment).

Only articles published in English language have been considered.

## Selection criteria for the studies

## Inclusion criteria

A study was considered eligible for inclusion in this systematic review if it met the following criteria:

1. Type of studies: Randomized controlled clinical trials
2. Subjects: Adults who were diagnosed with periodontitis and were systemically healthy and non-smoking at any age range.
3. Type of treatment intervention: Oral probiotic administration compared with placebo, or without any interventions. Randomized controlled clinical trials were included when they (1) tested one or more probiotic agents as an adjunct to scaling and root planing (SRP) and (2) had a control group that received the same SRP as the treatment group alone or with a placebo. We considered any type of probiotics with any type of administration method.
4. Types of outcome measures: reported results in terms of PPD, CAL and BOP with the minimum follow-up of 28 days.

## Exclusion criteria

Studies were excluded if they were animal experiments or in vitro studies, duplicated or affiliated studies, included patients with the habit of smoking or systemic disease, used the probiotics without SRP treatment; the data was incomplete or the full text was unavailable.

## Article review and data extraction

Titles and abstracts of potential publications were scanned by two blinded authors independently (G.H.Q and C.X) and further defined whether or not consistent to inclusion. When studies appeared to meet the inclusion criteria or

information in the title and abstract was insufficient to reach an explicit decision, full texts were reviewed.

Disagreements were resolved through discussions between authors and when still a consensus could not be reached, a third examiner (L. L) was consulted. Lack of pertinent data, the relevant details were provided by contacting the authors of the identified articles. Kappa values, reflecting an almost perfect inter-author agreement, were equal to 0.89 (p < 0.001).

Basic information extraction from papers included the year and authors of publication, the type of study design, the population demographics, the definition of the periodontitis, the probiotic bacterial strains used, also their mode of administration such as the frequency and duration, other associated treat models, the follow-up procedures, the adverse effects and the key parameters recorded, and the outcome assessments. (Fig. 1 and table 1)

## Search outcomes and evaluation

PPD, CAL and BOP parameters were evaluated as the primary outcomes. Secondary outcomes concluded plaque index (PI), gingival index (GI) or gingival bleeding index (GBI), oral malodor parameters, microbiological effects, the progression and prognosis of disease and the need for additional periodontal treatment. Analysis and recording were implemented independently by two authors (G.H.Q and C.X).

## Risk of bias assessment

Quality analysis according to the Cochrane Reviewers' Handbook [24] was performed independently by two reviewers (G.H.Q and C.X) assessing the risk of bias assessment which included seven criteria: random sequence generation, allocation concealment, blinding of participants and personnel, blinding of outcome assessment, incomplete outcome data, selective reporting and other sources of bias. Related one or more items could not be found in the included studies, the risk profile was defined as high risk; studies were defined as unclear risk if one or more criteria were partly met; the research depicted the study protocol (adequate randomization and allocation concealment, completeness of outcome data and blinding and no selective reporting and other sources of bias) detailedly, it was low risk. Discussing and resolving the differences within the reviewers.

## Data items

The meta-analysis integrated the average difference between baseline and follow-up (1 month, 3 months and 6 months) of PPD reduction (overall, stratified for moderate and deep pockets), CAL gain (overall, stratified for moderate and deep pockets) and reduction of percentage of sites with BOP (the whole-mouth).

## Data synthesis

First, Q and $I^2$ test was conducted to check the heterogeneity among studies. P value of Q statistic<0.1 or $I^2$ value higher than 40% was defined as an indicator of heterogeneity. Mean differences (MD) and 95% confidence interval (CI) were regarded as the differences of continuous outcomes between SRP + probiotic and SRP group for using either fixed or random models. Mean differences and standard errors of each included studies were enrolled. The mean difference could also be estimated by standard deviation using $\sigma d = sqrt\ (\sigma_1^2/n_1 + \sigma_2^2/n_2)$ formula since data was not presented in the form of mean differences. The meta-analysis were performed using Review Manager (Version 5.2. The Cochrane Collaboration, 2013, Oxford, UK).

# Results

## Study selection

Quickly browsing the search items, 291 potentially relevant papers were extracted. After moving the duplicates, 187 remained. After looking through the titles and abstracts, 169 articles were excluded owing to not fulfilling the inclusion criteria. Then evaluating carefully the 18 remaining articles, 7 were excluded for not confirming to the required intervention and data unavailable. (Appendix S1). Besides, two studies [25, 26] were performed at the same centre and on the same date. Hence, after discussion, it came to the conclusion that the participants enrolled in the study of İnce et al. were a subgroup of the participants in the study of Tekce et al. Thus, a total of eleven articles fulfilled the inclusion criteria and were concluded [25-35] and finally ten were further conducted a meta-analysis. [25, 27-35] (Fig. 1).

## Study characteristics

The studies included were all RCT published in the English language from 2010 to 2020 and the basic characteristics were demonstrated in table 1. All of them were designed for comparison between groups and were conducted at a single centre. All studies demonstrated the sample size calculation and all patients recruited were without systemic diseases, habit of smoking and also without history of antibiotic administration within 6 months before entering the experiment.

## Treatment modalities

Oral hygiene instructions and placebo control were provided in all studies. SRP was always performed with ultrasonic scalers and manual instruments. Lactobacillus reuteri [25, 26, 28, 31, 33, 35] two times per day, Lactobacillus rhamnosus SP1 [27] and Lactobacillus plantarum [30] one time per day, Lactobacillus Salivarius+Lactobacillus reuteri twice a day [30], L. salivarius NK02 twice a day [29], and Streptococcus oralis KJ3+Streptococcus uberis KJ2+Streptococcus rattus JH145 [32] twice a day, Bifidobacterium animalis subsp. Lactis (B. lactis) HN019 probiotic [34] were evaluated for their probiotic effect as adjuvant to periodontitis treatment. The organisms were orally administered as lozenges [25, 26, 28, 31, 33-35 ], tablets [32], sachet [27], mouthwashes [29, 30], drops [33] or solution [30]. The probiotic concentrations varied among $1´10^8$, $2´10^8$, $1×10^9$ colony forming units (CFUs) per day.

## Risk of bias across studies

All included studies in this systematic review were performed quality analysis according to the Cochrane Reviewers' Handbook (Higgins & Green 2011). All the included studies were doubled-blinded RCTs, and adopted computer-based randomization program, block randomization and randomization table. The allocation concealment, the shape of probiotics and placebo were identical envelopes, bottles, containers, and mouthwashes in all the included studies. All the outcome evaluators did not know the therapy protocol. Selective reporting and conflict of interests were not found in all studies [25-35]. Three researches [30, 33, 35] had lost interviews, one [30] was defined as unclear risk with the rate of follow-up below 10%, two were defined as high risk with the rate of follow-up more than 20% which could contribute to attribution bias and reporting bias [33, 35] (Figure 7). Overall, the literature included in this study was of good quality.

## The effect of probiotics in periodontitis

## The meta analysis of PPD reduction

Inter-study heterogeneity appeared significantly regarding overall PPD reduction at 1, 3 and 6 months (c2 =52.02, 36.62, 45.42, $I^2$ = 92%, 81%, 91% respectively, p< 0.00001). Inter-study heterogeneity appeared not significantly regarding PPD reduction in moderate and deep pockets at 3 months (c2 =7.93, 10.49, $I^2$ = 37%, 52% , p= 0.16, 0.06 respectively). The meta-analysis confirmed a significantly more overall PPD reduction at 1 month (0.48mm, 95% CI [-0.82, -0.14], p = 0.005) favoring the superior clinical efficacy of SRP+probiotics when compared to SRP+placebo treatment. Conversely, this advantage could not be implied at 3 and 6 months (0.14mm, 95% CI [-0.3, 0.01], p = 0.07; 0.2mm, 95% CI [-0.54, 0.14], p = 0.26 respectively) (Fig. 2A-C). When stratified for depth of pocket, there were a significantly more PPD reduction in both moderate (0.19mm, 95% CI [-0.27, -0.1], p< 0.00001) and deep pockets (0.58mm, 95% CI [-0.73, -0.43], p< 0.00001) at 3 months favoring the superior clinical efficacy of SRP+probiotics compared to SRP+placebo treatment (Fig. 4A, B).

## The meta analysis of CAL/attachment gain

Inter-study heterogeneity appeared significantly regarding overall CAL gain at 1, 3 and 6 months ($c2$ =7.88, 22.07, 153.84, $I^2$ = 62%, 69%, 97% respectively, p<0.05) and CAL gain in deep pockets at 3 months ($c2$ = 23.33, $I^2$ = 83%, p=0.0001). Inter-study heterogeneity appeared not significantly regarding CAL gain in moderate pockets at 3 months ($c2$ =6.51, $I^2$ = 39%, p= 0.16). The meta-analysis confirmed a significantly more overall CAL gain at 1 (0.35mm, 95% CI [-0.54, -0.16], p = 0.0004) and 3 months (0.13mm, 95% CI [-0.26, -0.01], p = 0.04) favoring the superior clinical efficacy of SRP+probiotics when compared to SRP+placebo treatment. Conversely, this advantage could not be implied at 6 months (0.19mm, 95% CI [-0.75, 0.38], p = 0.52) (Fig. 3A-C). When stratified for depth of pocket, there were a significantly more CAL gain in moderate (0.2mm, 95% CI [-0.3, -0.1], p=0.0001) not deep pockets (0.44mm, 95% CI [-0.99, 0.11], p=0.12) at 3 months favoring the superior clinical efficacy of SRP+probiotics compared to SRP+placebo treatment (Fig. 5A, B).

## The meta analysis of percentage reduction of BOP

Inter-study heterogeneity appeared significantly regarding overall BOP% reduction at short-term ($c2$ =114.23, $I^2$ =93%, p=0.001). Inter-study heterogeneity appeared not significantly regarding overall BOP% reduction at 6 months ($c2$ =7.49, $I^2$ = 47%, p= 0.11). The meta-analysis confirmed a significantly more BOP% reduction at short-term (10.38, 95% CI [-16.59, -4.16], p = 0.001) and 6 months (7.57, 95% CI [-8.64, -6.5], p< 0.00001) favoring the superior clinical efficacy of SRP+probiotics when compared to SRP+placebo treatment (Fig. 6A, B).

## GBI, GI and PI changes and other outcomes measures

Nine studies have evaluated the GBI, GI or PI changes and all proved significant improvement after both periodontal treatments [25-32, 35]. Three studies[25, 26, 28] recognized a significantly more reduction of PI, GI with SRP+probiotics protocol, whereas four studies did not conclude a significantly different change in PI in the SRP+probiotics group when compared to SRP+placebo group [27, 29, 32, 35]. Additionally, Teughels et al and Laleman et al corroborated that % of sites with supragingival plaque was significantly lower at 6 and 24 weeks respectively in test group, and only gingival index was significantly decreased in the test group compared that of

control group in the research of sajedinejad et al. Eight studies [34, 30, 32, 28, 29, 31, 32, 25] assessed the microorganism index. Invernici et al observed significantly lower mean cumulative proportions of orange (at 30 days) and red (at 90 days) complexes, significantly larger proportion of blue complex bacteria at 90 days and more pronounced reduction in the count of P. gingivalis, Treponema denticola, F. nucleatum, Campylobacter showae, and Eubacterium nodatum for deep periodontal pockets at 90 days in the SRP+probiotics group than in the SRP+placebo group (p < 0.05). This can also be found in the study of Penala et al, showing more reduction of red complex organisms at 1 month in test group. However Laleman et al (2020) had a contradictory finding that both treatment did not show any microbiological impact on the four studied micro-organisms (P. gingivalis, P. intermedia, F. nucleatum and A. actinomycetemcomitans). Interestingly, Vivekananda et al delineated that both treatment modalities can reduce the CFU counts of the pathogens A. actinomycetemcomitans, P. gingivalis, and P. intermedia by approximately 1 log10 unit from $10^6$ CFU/ml to less than $10^5$ CFU/ml. Sajedinejad et al demonstrated that the reduced bacterial colony count of A. actinomycetemcomitans in the test group was significantly less pronounced compared to that of the control group. Teughels et al and Laleman et al (2015) both manifested that in saliva, P. intermedia numbers in the SRP+Probiotics group were significantly (p < 0.05) lower at week 12 when compared to the SRP+placebo group. Additionally, Teughels et al further unleashed that there was significantly (p < 0.05) larger reductions in P. gingivalis numbers in the subgingival, supragingival and saliva samples in the SRP+Probiotics group over the 12 week period, when compared to the SRP+placebo group. Furthermore, Tekce et al stressed a significant decrease in proportions of obligate anaerobes at days 21, 90 and 180 of SRP+probiotics group. When considering the need of surgery which was defined as persisting pockets >4 mm with BOP [36], Tekce et al showed significant differences between groups (p < 0.05) in the percentage of sites and teeth as well as the number of patients needed for surgery through 1 year follow-up whereas Laleman et al (2020) only identified statistically significant differences at site level favoring the superior clinical efficacy of the SRP+probiotics treatment when compared to the SRP+placebo. Teughels et al stressed that only initially deep sites showed a significantly (p < 0.05) lower need for surgery at 12 weeks with significantly (p < 0.05) fewer patients classified as needing surgery on ≥3 teeth when they received SRP+probiotics treatment. Five included studies [25, 27, 31, 33, 34] in which the periodontal risk assessment tool [37] was conducted, reported that after receiving additional probiotics treatment, more patients were classified as low risk and fewer patients as high risk for disease progression. Moreover, the study of Penala et al demonstrated that there was a statistically significant reduction in halitosis scores (p < 0.05) at 1 month and 3 months in test group when compared to placebo. As for anti-inflammatory effects, Ince et al illustrated that decreased GCF MMP-8 levels and increased TIMP-1 levels were more significantly important up to days 180 (p<0.05) in experimental group. Invernici et al highlighted that test group had higher levels of IL-10 at 30 days (p < 0.05) and the control group had a higher ratio of IL-1β (at 30 and 90 days) and of IL-8 (at 30 days) (p < 0.05).

## Compliance and adverse effects of probiotics

None of the RCT included in this review reported the adverse effects of probiotic administration and patients

showed good compliance with probiotics application.

## Discussion

As the mainstay of the management of periodontitis is the reduction and elimination of specific periodontal pathogens (periodontopathogens/pathobionts) with SRP always being a primary step in the treatment [38], further antimicrobial agents can also be used in conjunction with mechanical procedures to reduce the pathogenic microbial burden and provide a satisfactory clinical outcome particularly in chronic situations [39, 40]. However, there are evidence that periodontopathogens, such as Tannerella forsythia and A. actinomycetemcomitans, remain in periodontal pockets after nonsurgical therapy [41, 42] ; bacterial recolonization occurs even shortly after scaling and root planning and the emergence of antibiotic resistance in these pathobionts have provoked human's concerns. Under these circumstances, it is urgently to call for new approaches for the management of periodontitis. These limitations of conventional periodontal therapy give rise to diverse innovative approaches that have been applied during the last few decades as an adjuvant treatment of periodontitis such as probiotics, so that the disease-causing pathogens are inhibited, promoting the development of a healthy flora, thus leading to restoration of health [43-45]. Probiotics therapy has gained great success in conquering intestinal inflammatory diseases and increasingly occupies the dominant position in selecting therapies to treat some certain intestinal abnormities [46]. On the basis that the oral cavity is closely connected with the intestinal canal and they share plenty of similarities, it is well-founded to postulate that the probiotics can also be applied to treat dental inflammatory diseases.

This systematic review and meta-analysis aimed to unveil whether adjunctive use of probiotics had additional clinical efficacy in the treatment of periodontitis. Our findings were in favor of administration of probiotics as an adjuvant to SRP compared to SRP therapy alone at short-time. All the eleven studies assessed had a low risk of bias and ten studies were incorporated in the meta-analysis. Moreover, the results showed significantly more overall PPD reduction at 1 month (0.48mm, p=0.005), overall CAL gain at 1 (0.35mm, p=0.004) and 3 months (0.13mm, p=0.04) and BOP percentage reduction (10.38, p=0.001) at short-term and 6 months (7.57, p< 0.00001) favoring SRP+probiotic treatment. Moreover, significantly more reduction of PPD for moderate (0.19mm, p< 0.00001), deep pockets (0.58mm, p< 0.00001), and gain of CAL for moderate pockets (0.20mm, p=0.0001) were observed at 3 months favoring adjunctive efficacy of probiotics without any adverse effects. However, there were no significant differences of overall PPD reduction at 3 (0.14mm, p=0.07) and 6 months (0.2mm, p=0.26) and overall CAL gain (0.19mm, p=0.53) at 6 months between two groups. This implied that adjunctive treatment with probiotics had prominent advantage in more PPD reduction (0.48mm), CAL gain (0.35mm), BOP% reduction (10.38%) at short-term and even significantly prominent BOP% improvements at 6 months when compared to SRP+placebo, which agreed nicely with one meta-analysis showing a range of average CAL improvements between 0.2 and 0.6 mm over SRP alone by means of scaling and root planing with or without adjuncts[47]. Additionally, the PPD improvement in moderate (mean 0.19mm) and deep pockets (0.58mm) and CAL improvement in moderate pockets (mean 0.2mm) over SRP+placebo could still be observed in SRP+probiotics treatment indicating a reduced need for additional periodontal therapies after SRP. Interestingly, Martin-Cabezas et al concluded that a statistically significant CAL gain (0.42 mm, p = 0.002) and BOP% reduction (14.66, p = 0.003) for SRP+probiotic treatment over SRP at short-term and overall PPD reduction could only be found in moderate (0.18mm, p = 0.001) and deep pockets (0.67mm, p < 0.001) [48]. We shared some similarities with this study and also discrepancies exit. The contradictions could be attributed to that this meta-analysis only included three studies with the same probiotics (Lactobacillus reuteri).

Considering that applying probiotics can result in the reduction of burden of periodontopathic organisms such as A. actinomycetemcomitans, P. intermedia and P. gingivalis [34, 30, 32, 28, 29, 31, 32, 25] and the concentrations of proinflammatory factors [26, 34], however, the mechanisms underlying the observed salutary effect of probiotics are unclear as yet. According to the ecological plaque hypothesis and the changes of the periodontal microenvironment [44], the possible mechanisms may be the potential of probiotics to change the overall composition of the periodontal biofilm in favor of commensals, alleviate the dysbiosis caused by periodontopathogens (the so called 'red complex bacteria') and competitively capture the adhesion sites and nutrients [49, 50]; to modulate the immune system [51, 52]; to modulate the cell proliferation and apoptosis [53]; to produce the antimicrobial substances, such as lactic acid, hydrogen peroxide, and reuterin [49, 54]; and to modulate the pH and/or the oxidation of the microenvironment [55]. Further fundamental researches are needed to shove away these puzzles and provide profound theory for better understanding the functions of probiotics.

Now that probiotics have potential in improving clinical performances and bringing the same clinical outcomes as other adjunctive periodontal treatment such as antibiotics and lasers, of both which have certain adverse effects, it is worthy to make it a regular periodontal therapy. However, it is disappointing to see that the probiotics therapy investigated in the selected studies varies in forms of administration, dosage, frequency and duration, and patients are all systematically healthy and nonsmokers. Hence, future studies should be agreed on a standardized protocol, such as the period, the dosage, the frequency and the form of probiotic administration so as to yield more predictable clinical outcomes, and whether the clinical advantage of probiotics can also be applied to periodontitis with systemic disease such as diabetes, atherosclerosis, inflammatory bowel disease, Alzheimer's disease and also in smokers. Additionally, since the adjunctive probiotics treatment on periodontal disease has dominant transient effects, the longer effects are little known and the recovery of oral microbiome after stopping probiotics is unknown which portray a high need for future investigations.

## Study limitations

This systematic review and meta-analysis included rigorous inclusion/exclusion criteria and an analysis of risk assessment. However, there still existed some limitations. The included studies are all in English with small sample size. Moreover, the definition of periodontitis, treatment protocols (split-mouth/parallel, the amount and type of probiotics, the formalization of probiotics and the sessions of SRP ), and the follow-up appointments (from 42 days to 360 days) varied among the included studies (Table 1).

# Conclusions

To conclude, under the limitations of the restricted RCTs regarding to the the effect of adjunctive probiotics to SRP in the management of periodontitis, our systematic review and meta-analysis manifests that adjunctive use of probiotics to SRP promises superior clinical benefits in more overall PPD reduction at 1 month, reducing PPD in moderate and deep pockets at 3 months, more overall CAL gain at 1 and 3 months, more CAL gain in moderate

pockets at 3 months, more BOP% reduction at short-term and 6 months, balancing periodontal pathogenic bacteria, controlling inflammation, reducing the need for surgery and improving the periodontal risk profile under the condition of systemically healthy and nonsmokers. As probiotic therapy could be an attractive supplementary adjunct for traditional therapies in managing periodontal disease, further long-term RCTs with large sample are warranted.

## Additional File Information

Additional file 1: PRISMA 2009 Checklist DOCK 16.3KB

Additional file 2: **Table 1**. Characteristics of included studies DOCK 30.6KB

Additional file 3: **Fig. 1** Flow chart of the study; **Fig. 2** (A) Forest plot of overall PPD reduction at 1 month; (B) Forest plot of overall PPD reduction at 3 months; (C)  Forest plot of overall PPD reduction at 6 months; **Fig. 3** (A) Forest plot of overall CAL gain at 1 month; (B) Forest plot of overall CAL gain at 3 months; (C) Forest plot of overall CAL gain at 6 months; **Fig. 4** (A) Forest plot of PPD reduction at 3 months in moderate pockets; (B) Forest plot of PPD reduction at 3 months in deep pockets; **Fig. 5** (A) Forest plot of CAL gain at 3 months in moderate pockets; (B) Forest plot of CAL gain at 3 months in deep pockets; **Fig. 6** (A) Forest plot of overall percentage of BOP reduction at short-term; (B) Forest plot of overall percentage of BOP reduction at 6 months; **Fig. 7** Summary of risk assessment PPTX 230.4KB

Supplementary file 1: Reasons of studies excluded DOCK 12.8KB

## Abbreviations

SRP, scaling and root planning; PPD, periodontal probing depth; BOP, bleeding on probing; CAL, clinical attachment level; CP, chronic periodontitis; AgP, aggressive periodontitis; AAP, advanced adult periodontitis; FMD, full-mouth disinfection; GBI, Gingival Bleeding Index; GI, Gingival Index; M/F, Male/Female; N, Population; OHI, oral hygiene instructions; PI, Plaque Index; PDI, Periodontal disease index;  RCT, randomized clinical trial; REC, gingival recession; MGI, modified gingival index; BANA, N-benzoyl-DL-arginine-naphthylamide; ORG, organoleptic scores; NK, not known; L, low risk bias; CI, confidence interval;  CFUs, colony forming units; $\sigma_d$, the standard deviation of mean difference; $\sigma_1$, standard deviation of baseline; $n_1$, participants of baseline; $\sigma_2$, standard deviation of follow-up; $n_2$, participants of follow-up

## Declarations

### Availability of data and materials

All data generated or analyzed during this study are included in this published article.

## Authors' contributions

HG, XC, LL, XW and YX adapted the economic model for this analysis. HG and XC wrote the first and subsequent versions of the manuscript. All the authors contributed to fruitful discussion of the results and to the review of the manuscript. YX is the guarantor of the overall content of the paper. All authors have read and approved the manuscript, and ensure the integrity and accuracy.

## Ethics approval and consent to participate

No applicable

## Consent for publication

No applicable

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## Conflict of interest

## Author details

1 Jiangsu Key Laboratory of Oral Diseases, Nanjing Medical University, Nanjing, People's Republic of China;

2 Department of Periodontics, The Affiliated Stomatological Hospital of Nanjing Medical University, Nanjing, People's Republic of China;

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# Table

**Table 1** Characteristics of included studies

| / on | Type | Clinical parameters | N M/F Age | CP definition | Treatment | Probiotic Administration | Adverse effects | Follow-up | Findings |
|---|---|---|---|---|---|---|---|---|---|
| kananda (2010) | RCT Placebo Parallel | PI, GI, GBI, PPD, attachment level (Indexed teeth of the Community Periodontal Index) | 30 CP 19/11 34–50 | Clinically evidenced by gingivitis together with mild to moderate periodontal pockets (5–7 mm) clinically and radiographic evidence of bone loss | OHI + SRP in 2 quadrants in both groups and in one session (the other 2 quadrants where left untreated). After 21 days: Placebo (control) Probiotic (test) | Lactobacillus reuteri 2 lozenges twice daily from day 21 to 42 1×108 CFU per lozenge | No | 42 days (days 21, 42) | PI, GI and GBI: Maximum reduction with SRP + probiotic protocol at day 42 (p < 0.001) . PPD: Maximum reduction at day 42 with SRP + probiotic protocol: from 5.08 (±0.75) to 3.78 (±0.61) mm. Significantly better than SRP alone (p < 0.001) . CAL: Best result at 42 days with SRP + probiotic (p < 0.001) from 3.93 (±0.93) to 2.85 (±0.71) mm . |
| et al. ) [26] | RCT Placebo Parallel | PI, GI, BOP, PPD, attachment level 6 sites/tooth | 30 CP 17/13 35–50 | Radiographically detected horizontal bone loss with presence of at least 2 teeth with one approximal site with PPD of 5–7 mm and a GI of ≥2 in each quadrant | OHI (2 groups) SRP + placebo (control) SRP + probiotic (test) (2 sessions of SRP) | Lactobacillus reuteri 2 lozenges per day for 3 weeks Started at onset of initial therapy 1×108 CFU per lozenge | No | 1 year (days 21, 90, 180, 360) | PI, GI and BOP were significantly lower in the test group compared with control group at all time points (p <0.05). PPD: Reduction of 1.70 mm in the test group and of 0.55 mm for the control (p = 0.001) at day 360. Attachment gain: 1.39 mm (test group) and 0.43 mm (control group) at day 360. Significant difference between groups (p = 0.001). |
| hels et al. 013) | RCT Placebo | PI, GI, BOP, PPD, REC, | 30 CP 15/15 | Severe generalized adult periodontitis (Van | OHI (2 groups) | Lactobacillus reuteri 2 lozenges per | No | 12 weeks | % of sites with supragingival plaque: Tended to be lower in |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | Parallel | attachment level | 45.73/46.6 | der Velden, U., 2005):≥14 teeth affected and bone loss >1/2 of the root length or attachment loss ≥6 mm | SRP (FMD) + placebo (control) SRP (FMD) + probiotic (test) | day for 12 weeks 1×108 CFU per lozenge | | (weeks 3, 6, 9, 12) | test group at 3 weeks (p = 0.089) and it was significantly lower at 6 weeks (p = 0.029). % of sites with gingival bleeding: Tended to be lower in test group at 3 and 6 weeks (p = 0.074 and 0.089 respectively) and it was significantly lower at 12 weeks (p < 0.0001). PPD: No significant differences between groups in PPD reduction at 12 weeks (p = 0.097). Significant larger reduction in moderate and deep pockets (p < 0.05) and lower % of deep pockets at 12 weeks for test group (p < 0.05). CAL: Significantly (p < 0.05) greater gain in CAL for the test group both in initially moderate and deep pockets at 12 weeks. REC: test group tended to show less recession formation (p = 0.089) at 12 weeks |
| les et 016) | RCT Placebo | PI, GI, BOP, PPD, | 28 CP 14/14 | Chronic periodontitis was | OHI (2 groups) | Lactobacillus rhamnosus SP1 1 sachet taken | No | 1 year (months 0, 3, 6, 9, 12) | There was a significant PPD reduction in both groups (p |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | Parallel | attachment level.<br><br>6sites/tooth | 36-60 | defined as having at least five teeth with periodontal sites with pocket probing depth (PPD) ≥ 5mm and clinical attachment loss (AL) ≥ 3 mm, 20% bleeding on probing (BOP) and extensive radiographically determined bone loss | SRP + placebo (control)<br><br>SRP + probiotic (test)<br><br>(4-6 sessions of SRP) | orally every day for 3 months<br><br>Started at the last session of SRP<br><br>2´107 CFU per sachet | | | < 0.05). For both shallow sites and moderate pockets, there was a significant reduction in PPD mean in both groups (p < 0.05).<br><br>Significantly lower percentage of sites and teeth with PPD ≥ 5mm in both groups (p < 0.05) were observed. However, there were no differences between groups (p > 0.05)<br><br>There were significant fewer patients with PPD ≥ 6 mm in test group (p < 0.05)<br><br>Statistically significant intra-group differences were observed in the amount of full- mouth CAL and PI reduction (p < 0.05). |
| la et al. ) [30] | RCT<br><br>Placebo<br><br>Parallel | PI, MGI, BI, BOP, PPD, attachment level, BANA,ORG.<br><br>6sites/tooth | 29CP<br><br>NK<br><br>25-59 | chronic periodontitis clinically evident with at least 4 teeth showing probing depth (PD) ≥ 5 mm,<br><br>clinical attachment<br><br>level (CAL) ≥4 mm | OHI (2 groups)<br><br>SRP + placebo (control)<br><br>SRP + probiotic (test)<br><br>(2 sessions of SRP) | Probiotic (Lactobacillus<br><br>Salivarius and Lactobacillus reuteri) mouthwash for 15 days after SRP and underwent subgingival delivery of probiotic solution at baseline (immediately after SRP), 1 | No | 3 months (months 0, 1, 3) | Treatment resulted in significant reduction in PD from baseline to 3 months in both groups (P < 0.05), but intergroup comparisons revealed no statistically significant difference in the PDR. However, test group showed lesser PD at 3 |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | | week, 2 weeks, and 4 weeks

2´109 CFU per strain | | | months when compared to control group. Test group showed statistically significant (P < 0.05)

PDR for moderate pockets. Both groups showed significant CAG at 3 months visit when compared to baseline, there were no differences between groups (p > 0.05) |
| linejad (2017) | RCT
Placebo
Parallel | PI, PDI, GI, BOP, PPD

6sites/tooth | 20CP

6/14

31-59 | moderate to severe chronic periodontitis with PPD ≥4 mm, CAL ≥3 mm, Bone loss ≥3 mm | OHI (2 groups)

SRP + placebo (control)

SRP + probiotic (test) | Twenty milliliters of L. salivarius NK02 mouthwashes was used twice a day for 28 days

Started at onset of initial therapy

1´108 CFU | No | 28 days (days 0, 14, 28) | BOP and PPD and gingival index were significantly decreased in the test group compared to the control group. (P value <0.05)

Changes in PDI (periodontal disease index) and PI were not significantly different. (P value >0.05) |
| nan (2015) | RCT
Placebo
Parallel | PI, GI, REC☐ BOP, PPD

6sites/tooth | 48AAP

26/22

37-58 | With moderate (initial PPD between 4 and 6 mm) and

deep (initial PPD ≥ 7 mm) pockets | OHI (2 groups)

SRP + placebo (control)

SRP + probiotic (test)

(1 session of SRP) | probiotic tablet containing Streptococcus Oralis KJ3, Streptococcus uberis KJ2 and Streptococcus rattus JH145 dissolve on their tongue twice a day for 3 months.Started at onset of initial therapy

1´108 CFU per strain | No | 24 weeks (weeks 0, 4, 8, 12, 24) | The PI, GI, REC, BOP, PPD measures were significantly (p < 0.05) improved at the 12- and the 24- week evaluation in both groups. However, no significant inter-group differences could be detected at any time point, except from the % of sites with plaque that were significantly lower in the probiotic |

| Study | Design | Parameters | Sample | Inclusion criteria | Groups | Probiotic | | Duration | Results |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | | group than in the control group at the 24-week evaluation |
| e et al. ) [25] | RCT Placebo Parallel | PI, GI, BOP, PPD, attachment level | 40 CP 18/22 35–50 | Radiographically detected horizontal bone loss with presence of at least 2 teeth with one approximal site with PPD of 5–7 mm and a GI of ≥ 2 in each quadrant | OHI (2 groups) SRP + placebo (control) SRP + probiotic (test) (2 sessions of SRP) | Lactobacillus reuteri 2 lozenges per day for 3 weeks Started at onset of initial therapy 1´108 CFU per lozenge | No | 1 year (days 21, 90, 180, 360) | PI, GI and BOP were significantly (p < 0.005) lower in the test group compared with control group at all time points. PPD : Reduction of 1.74 mm in the test group and of 0.57 mm for the control group (p = 0.001) at day 360. Attachment gain: 1.39 mm (test group) and 0.53 mm (control group) at day 360. Significant difference between groups (p = 0.001) |
| nici et 018) | RCT Placebo Parallel | PI, BOP, PPD, REC, attachment level 6sites/tooth | 41CP 20/21 | 30% or more of the sites with probing pocket depth (PPD) ≥ 4 mm and CAL ≥ 4 mm, presence of bleeding on probing (BOP) and a minimum of five teeth with at least one site with CAL and PPD ≥ 5 mm | OHI (2 groups) SRP + placebo (control) SRP + probiotic (test) (1 session of SRP) | Bifidobacterium animalis subsp. Lactis (B. lactis) HN019 probiotic 1 lozenge twice a day for 30 days (immediately after SRP) 1×109 CFU per lozenge | No | 90 days (days 0, 30, 90) | In moderate and deep pockets, the Test group had larger clinical attachment gain and lower PPD than the Control group at 90 days (p < 0.05). The Control group had a higher number of moderate (at 30 and 90 days) and deep (at 90 days) pockets than the Test group (p < 0.05). 55% of the patients in the Test group were at a low risk, only 30% of the patients in the Control group were |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | | classified as such. |
| | | | | | | | | | The test group had a lower rate of patients in need for additional periodontal treatment on more than three sites when compared to the Control group at 90 days (p < 0.05). |
| ...os et ...) [35] | RCT Placebo Parallel | PPD, BOP,visible plaque, attachment level 6sites/tooth | 41CP 15/26 53.3 mean | With at least 2 non-adjacent teeth with PPD ≥ 5mm and evidence of radiographic bone loss | OHI (2 groups) SRP + placebo (control) SRP + probiotic (test) (≥5 sessions of SRP) | Lactobacillus reuteri 2 lozenges twice a day for 28 days 1´108 CFU per lozenge | No | 180 days (days 0, 90, 180) | Among the test and control groups, there were significant intra-group differences in primary outcomes: CAL (both, p<0.001) and PPD (both, p<0.001); and in secondary outcomes: percentage of sites with 'bleeding on probing' (both, p<0.001) and visible plaque (both, p<0.001). No statistically significant inter-group differences in any outcomes at any time points (all, p>0.05) nor in the changes in outcomes (Δ) with time (all, p>0.05). |
| | | | | | | | | | There was a trend of a greater magnitude of statistical change occurring among the test group compared to the control group. |
| ...nan et | RCT | PPD, REC, | 39CP | Scaling and root | OHI (2 | Lactobacillus | No | 24 | After 24 weeks |

| | Placebo | attachment level, Full-Mouth Plaque Scores (FMPS), Full-Mouth Bleeding Scores (FMBS) 6sites/tooth | 27/12 34-83 | planing for moderate to severe chronic periodontitis should have been carried out at least 3 months and maximum 6 months ago and residual pockets should still be present; residual pockets were defined as pockets ≥ 6mm or pockets of 5mm with bleeding on probing; there had to be at least one residual pocket in two contralateral quadrants | groups) SRP with probiotic or placebo drops applied once split-mouth (1 session of SRP) Patients afterwards received lozenges (containing either probiotics or placebo) to use | reuteri 2 lozenges twice a day for 12 weeks ≥ 2×108 CFU per lozenge | weeks (weeks 0, 12, 24) | the overall PPD in the probiotic lozenges group (2.64±0.33mm) was significantly lower compared to the control lozenges (2.92±0.42mm). This difference was even more pronounced in moderate (4-6mm) and deep (≥7mm) pockets. In the probiotic lozenges group, there were also significantly more pockets converting from ≥4mm at baseline to ≤3mm at 24 weeks (67±18% versus 54±17%) and less sites in need for surgery (4±4% versus 8±6%) |
|---|---|---|---|---|---|---|---|---|
| )) [33] | Parallel | | | | | | | |

*BOP*, bleeding on probing; *CAL*, clinical attachment level; *CP*, chronic periodontitis; *AgP*, aggressive periodontitis; *AAP*, advanced adult periodontitis; *FMD*, full-mouth disinfection; *GBI*, Gingival Bleeding Index; *GI*, Gingival Index; *M/F*, Male/Female; *N*, Population; *OHI*, oral hygiene instructions; *PI*, Plaque Index; *PDI*, Periodontal disease index; *PPD*, periodontal probing depth; *RCT*, randomized clinical trial; *REC*, gingival recession; *MGI*, modified gingival index; *BANA*, N-benzoyl-DL-arginine-naphthylamide; *ORG*, organoleptic scores; *NK*, not known

# Figures
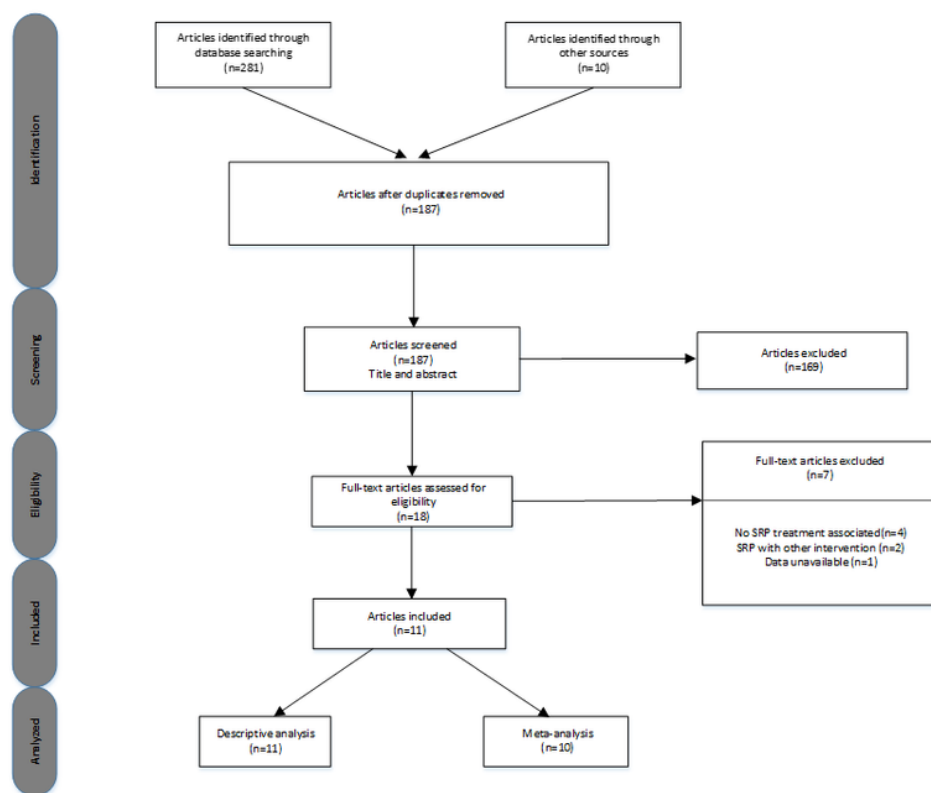
Figure 1

Flow chart of the study

Figure 2

**A**

| Study or Subgroup | Experimental Mean | SD | Total | Control Mean | SD | Total | Weight | Mean Difference IV, Random, 95% CI |
|---|---|---|---|---|---|---|---|---|
| Invernici 2018 | -0.48 | 0.3 | 20 | -0.32 | 0.27 | 21 | 20.8% | -0.16 [-0.33, 0.01] |
| Sajedinejad 2017 | -1.11 | 0.23 | 10 | -0.22 | 0.38 | 10 | 19.2% | -0.89 [-1.17, -0.61] |
| Tekce 2015 | -1.44 | 0.33 | 20 | -0.85 | 0.32 | 20 | 20.4% | -0.59 [-0.79, -0.39] |
| Teughels 2013 | -1.41 | 0.25 | 15 | -1.39 | 0.15 | 15 | 21.2% | -0.02 [-0.17, 0.13] |
| Vivekananda 2010 | -1.31 | 0.49 | 15 | -0.49 | 0.39 | 15 | 18.4% | -0.82 [-1.14, -0.50] |
| **Total (95% CI)** | | | 80 | | | 81 | 100.0% | **-0.48 [-0.82, -0.14]** |

Heterogeneity: Tau² = 0.13; Chi² = 52.02, df = 4 (P < 0.00001); I² = 92%
Test for overall effect: Z = 2.81 (P = 0.005)

Favours [experimental]   Favours [control]

**B**

| Study or Subgroup | Experimental Mean | SD | Total | Control Mean | SD | Total | Weight | Mean Difference IV, Random, 95% CI |
|---|---|---|---|---|---|---|---|---|
| Invernici 2018 | -0.52 | 0.27 | 20 | -0.25 | 0.22 | 21 | 13.3% | -0.27 [-0.44, -0.10] |
| Laleman 2015 | -1.35 | 0.3 | 24 | -1.34 | 0.33 | 24 | 13.1% | -0.01 [-0.19, 0.17] |
| Laleman 2019 | -0.43 | 0.23 | 19 | -0.44 | 0.28 | 20 | 13.5% | 0.01 [-0.15, 0.17] |
| Morales 2016 | -0.5 | 0.2 | 14 | -0.4 | 0.4 | 14 | 11.7% | -0.10 [-0.33, 0.13] |
| Pelekos 2019 | -0.4 | 0.2 | 21 | -0.5 | 0.3 | 20 | 13.6% | 0.10 [-0.06, 0.26] |
| Penala 2016 | -0.76 | 0.43 | 15 | -0.69 | 0.38 | 14 | 10.1% | -0.07 [-0.36, 0.22] |
| Tekce 2015 | -1.44 | 0.33 | 20 | -0.85 | 0.32 | 20 | 12.5% | -0.59 [-0.79, -0.39] |
| Teughels 2013 | -0.99 | 0.22 | 15 | -0.76 | 0.36 | 15 | 12.2% | -0.23 [-0.44, -0.02] |
| **Total (95% CI)** | | | 148 | | | 148 | 100.0% | **-0.14 [-0.30, 0.01]** |

Heterogeneity: Tau² = 0.04; Chi² = 36.62, df = 7 (P < 0.00001); I² = 81%
Test for overall effect: Z = 1.79 (P = 0.07)

Favours [experimental]   Favours [control]

**C**

| Study or Subgroup | Experimental Mean | SD | Total | Control Mean | SD | Total | Weight | Mean Difference IV, Random, 95% CI |
|---|---|---|---|---|---|---|---|---|
| Laleman 2015 | -1.52 | 0.38 | 24 | -1.62 | 0.41 | 24 | 20.3% | 0.10 [-0.12, 0.32] |
| Laleman 2019 | -0.45 | 0.2 | 19 | -0.36 | 0.26 | 20 | 21.4% | -0.09 [-0.24, 0.06] |
| Morales 2016 | -0.6 | 0.3 | 14 | -0.4 | 0.4 | 14 | 19.7% | -0.20 [-0.46, 0.06] |
| Pelekos 2019 | -0.5 | 0.3 | 21 | -0.7 | 0.5 | 20 | 19.8% | 0.20 [-0.05, 0.45] |
| Tekce 2015 | -1.77 | 0.69 | 20 | -0.7 | 0.24 | 30 | 18.8% | -1.07 [-1.38, -0.76] |
| **Total (95% CI)** | | | 98 | | | 108 | 100.0% | **-0.20 [-0.54, 0.14]** |

Heterogeneity: Tau² = 0.14; Chi² = 45.42, df = 4 (P < 0.00001); I² = 91%
Test for overall effect: Z = 1.14 (P = 0.26)

Favours [experimental]   Favours [control]

Figure 2

(A) Forest plot of overall PPD reduction at 1 month; (B) Forest plot of overall PPD reduction at 3 months; (C) Forest plot of overall PPD reduction at 6 months

## Figure 3



## Figure 3

(A) Forest plot of overall CAL gain at 1 month; (B) Forest plot of overall CAL gain at 3 months; (C) Forest plot of overall CAL gain at 6 months
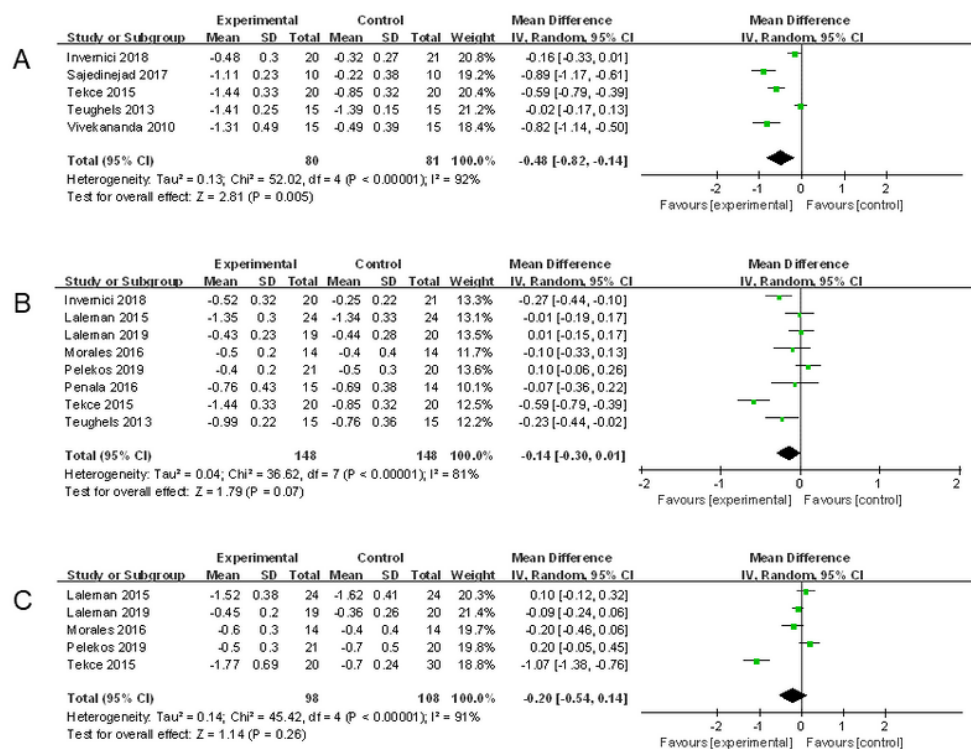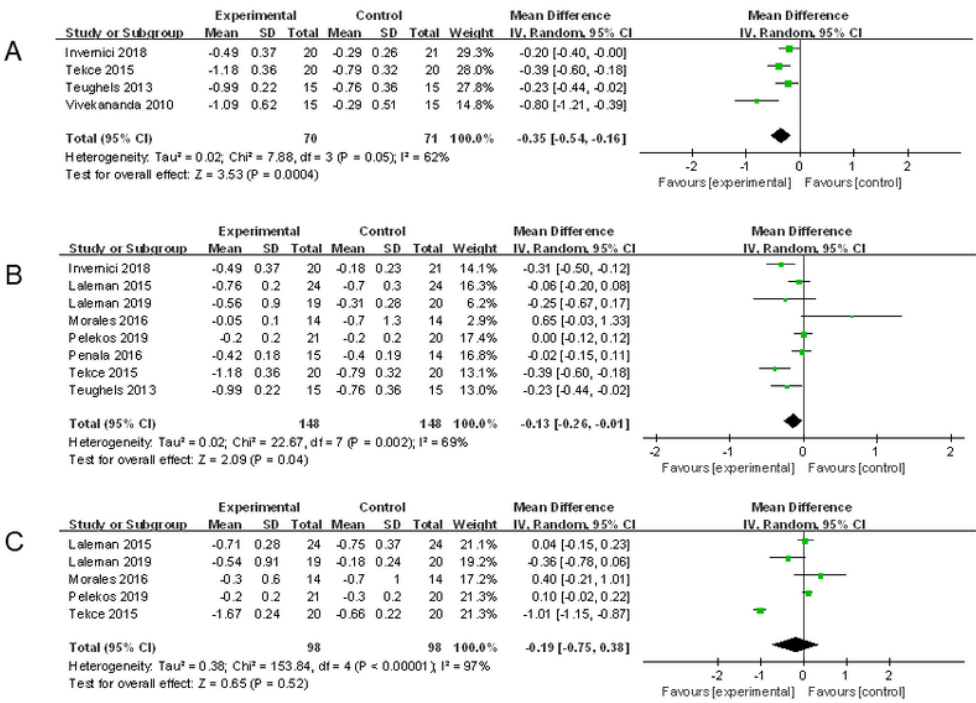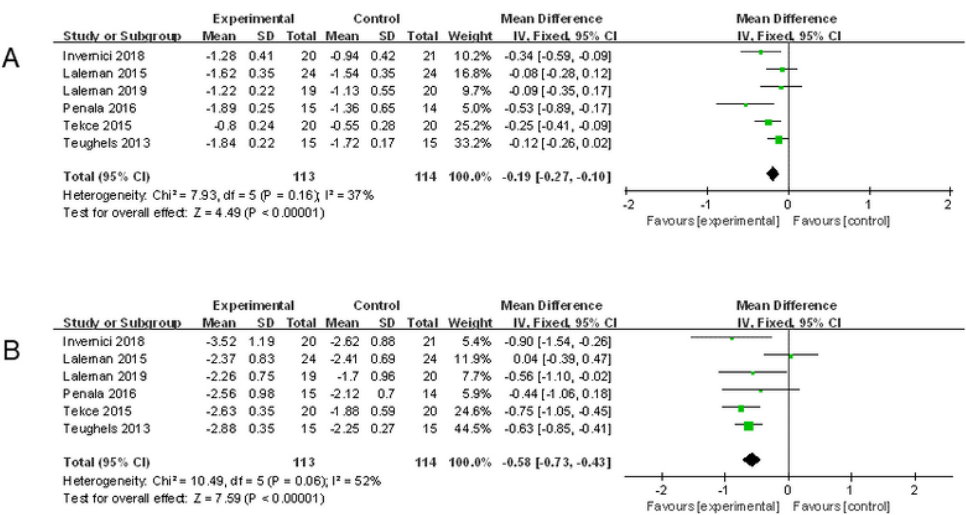
# Figure 4



## Figure 4

(A) Forest plot of PPD reduction at 3 months in moderate pockets; (B) Forest plot of PPD reduction at 3 months in deep pockets
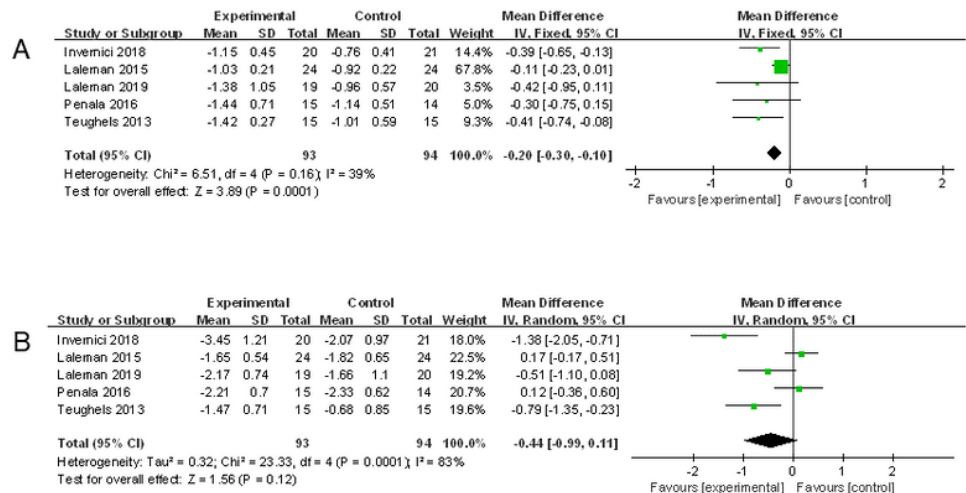
# Figure 5

# Figure 5

(A) Forest plot of CAL gain at 3 months in moderate pockets; (B) Forest plot of CAL gain at 3 months in deep pockets

## Figure 6



**A**

| Study or Subgroup | Experimental Mean | SD | Total | Control Mean | SD | Total | Weight | Mean Difference IV, Random, 95% CI |
|---|---|---|---|---|---|---|---|---|
| Invernici 2018 | -14.55 | 8.63 | 20 | -11.04 | 10.35 | 21 | 12.3% | -3.51 [-9.33, 2.31] |
| Laleman 2015 | -60 | 12 | 24 | -57 | 9 | 24 | 12.2% | -3.00 [-9.00, 3.00] |
| Laleman 2019 | -14 | 11 | 19 | -13 | 10 | 20 | 11.9% | -1.00 [-7.61, 5.61] |
| Morales 2016 | -12.9 | 15.9 | 14 | -10.3 | 14.6 | 14 | 9.5% | -2.60 [-13.91, 8.71] |
| Pelekos 2019 | -22.1 | 26.3 | 21 | -26.8 | 20.2 | 20 | 8.0% | 4.70 [-9.61, 19.01] |
| Sajedinejad 2017 | -49.46 | 5.82 | 10 | 2.23 | 23.84 | 10 | 7.6% | -51.69 [-66.90, -36.48] |
| Tekce 2015 | -72.25 | 1.95 | 20 | -66.8 | 1.28 | 20 | 13.7% | -5.45 [-6.47, -4.43] |
| Teughels 2013 | -55.19 | 4.85 | 15 | -50.95 | 4 | 15 | 13.3% | -4.24 [-7.42, -1.06] |
| Vivekananda 2010 | -70.4 | 10.7 | 15 | -32.5 | 10 | 15 | 11.5% | -37.90 [-45.31, -30.49] |
| **Total (95% CI)** | | | 158 | | | 159 | 100.0% | -10.38 [-16.59, -4.16] |

Heterogeneity: Tau² = 73.03; Chi² = 114.23, df = 8 (P < 0.00001); I² = 93%
Test for overall effect: Z = 3.27 (P = 0.001)

**B**

| Study or Subgroup | Experimental Mean | SD | Total | Control Mean | SD | Total | Weight | Mean Difference IV, Fixed, 95% CI |
|---|---|---|---|---|---|---|---|---|
| Laleman 2015 | -47 | 17 | 24 | -48 | 19 | 24 | 1.1% | 1.00 [-9.20, 11.20] |
| Laleman 2019 | -16 | 8 | 19 | -11 | 10 | 20 | 3.6% | -5.00 [-10.67, 0.67] |
| Morales 2016 | -11.3 | 14.4 | 14 | -5.9 | 14.9 | 14 | 1.0% | -5.40 [-16.25, 5.45] |
| Pelekos 2019 | -29.9 | 15.2 | 21 | -32.4 | 19 | 20 | 1.0% | 2.50 [-8.06, 13.06] |
| Tekce 2015 | -76.6 | 2.1 | 20 | -68.7 | 1.4 | 20 | 93.4% | -7.90 [-9.01, -6.79] |
| **Total (95% CI)** | | | 98 | | | 98 | 100.0% | -7.57 [-8.64, -6.50] |

Heterogeneity: Chi² = 7.49, df = 4 (P = 0.11); I² = 47%
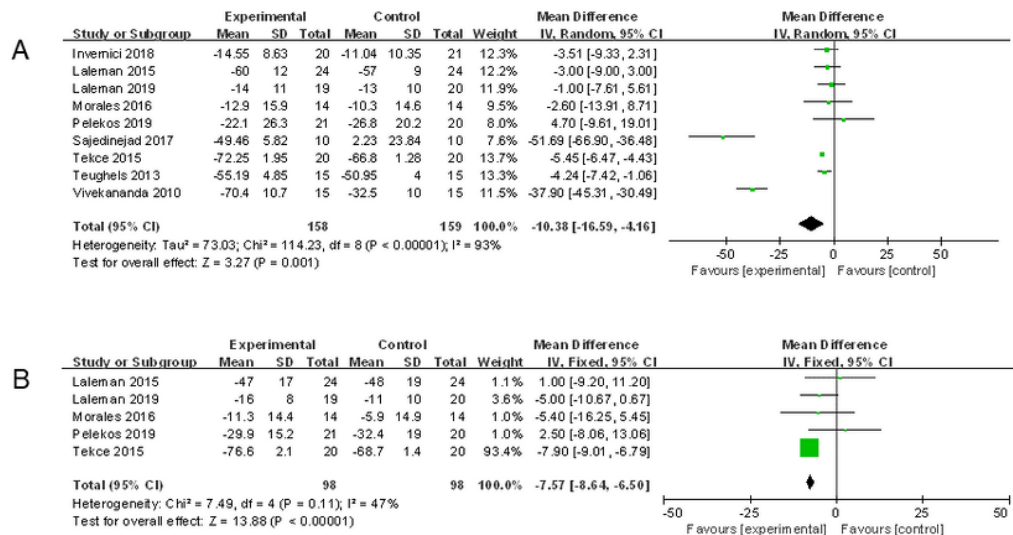Test for overall effect: Z = 13.88 (P < 0.00001)

# Figure 6

(A) Forest plot of overall percentage of BOP reduction at short-term; (B) Forest plot of overall percentage of BOP reduction at 6 months
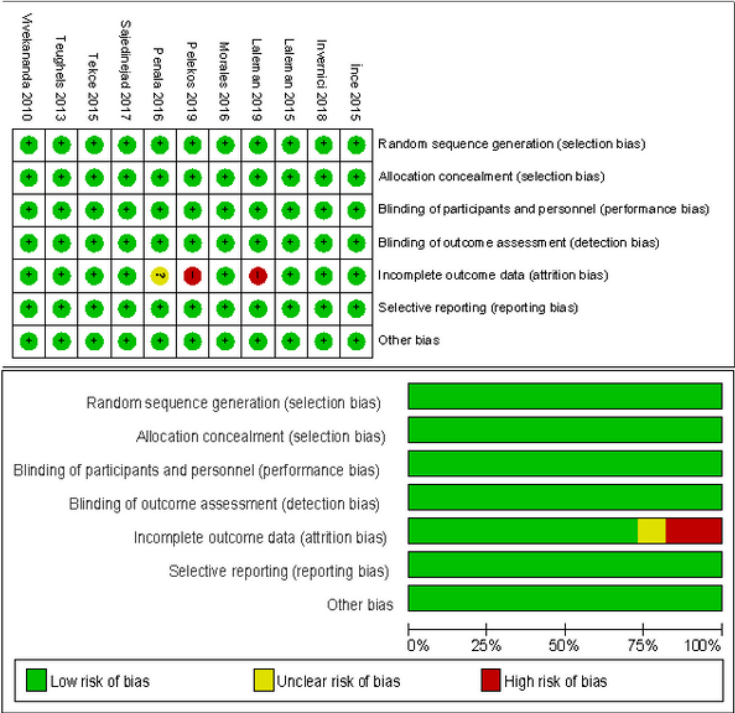
Figure 7



**Figure 7**

Summary of risk assessment graph. Green, yellow and red refer to low risk of bias, unclear risk of bias and high risk of bias respectively.

# Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- PRISMA2009Checklist.docx