Systemaic Review
Periodontics

Microbiological outcomes from different periodontal maintenance interventions: a systematic review

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Abstract: This study aimed to investigate the differences in the subgingival microbiological outcomes between periodontal patients submitted to a supragingival control (SPG) regimen as compared to subgingival scaling and root planing performed combined with supragingival debridement (SPG + SBG) intervention during the periodontal maintenance period (PMP). A systematic literature search using electronic databases (MEDLINE and EMBASE) was conducted looking for articles published up to August 2016 and independent of language. Two independent reviewers performed the study selection, quality assessment and data collection. Only human randomized or non-randomized clinical trials with at least 6-months-follow-up after periodontal treatment and presenting subgingival microbiological outcomes related to SPG and/or SPG+SBG therapies were included. Search strategy found 2,250 titles. Among these, 148 (after title analysis) and 39 (after abstract analysis) papers were considered to be relevant. Finally, 19 studies were selected after full-text analysis. No article had a direct comparison between the therapies. Five SPG and 14 SPG+SBG studies presented experimental groups with these respective regimens and were descriptively analyzed while most of the results were only presented graphically. The results showed that both SPG and SPG+SBG protocols of PMP determined stability in the microbiological results along time. Nevertheless, new studies comparing these interventions in PMP are needed, especially if the limitations herein discussed could be better controlled.

Keywords: Dental Plaque; Microbiology; Root Planing; Review.

Introduction

The literature has clearly shown that the maintenance of periodontal results after an adequate therapy is delivered depends on the quality of the supragingival plaque control (SPG).[1,2,3,4,5] However, during the past 20 years the subgingival scaling and root planing (SBG) has been associated to that control and this association (SPG+SBG) been recognized as the choice therapy during periodontal maintenance period (PMP).[6,7,8] Nevertheless, studies comparing this combination to other protocols were not available until recently when a clinical study[9] and two systematic reviews[10,11] suggested that the SPG biofilm control alone might be the choice during PMP. The

authors showed no clinical outcomes benefits from the addition of the subgingival scaling (SPG + SBG).

Microbiological composition of the subgingival area after therapy as well is an issue of discussion. It is suggested that microbiological differences between biofilms may explain part of the failures observed along PMP period. The impact that the subgingival instrumentation has over the amount and composition of the biofilm has been shown.[12] However, not only the subgingival intervention but also the supragingival control modifies the quality and quantity of the subgingival biofilm.[13,14,15] Besides, evidences support the biofilm as the main source of bacteria to the subgingival area.[16] So, in theory, both interventions, singly, would be able to maintain a microbiota compatible with periodontal stability and health along years. Taking this plausibility in mind, this systematic review aimed at investigating how does the subgingival microbiota respond to the SPG in comparison with the SPG+SBG as maintenance protocols.

Methodology

This systematic review was conducted in accordance with the guidelines of the Preferred Reporting Items for Systematic and Meta-Analysis (PRISMA) Statement[17] and used the reference manager Mendeley (version Desktop 1.16.1-OSX-Universal; Elsevier Inc., New York, USA). Neither a protocol nor a systematic review registration was considered.

Focused question

In periodontal patients submitted to PMP, is there a difference in the subgingival microbiological outcomes from the SPG regimen as compared to the SPG+SBG intervention?

Search strategy

Studies were identified by electronic databases (MEDLINE via PubMed, and EMBASE) searching for studies published up to August 2016. No language restrictions were applied. The following MesH terms were used in different arrangements (Appendix 1-A; 1-B): *randomized clinical trial; randomized controlled trial; clinical trial; longitudinal study; prospective study*; *supportive periodontal care; periodontal maintenance*, and *microbiology*.

A manual search of the reference list from narrative and systematic reviews studies, as well as the bibliographies of the included studies, was performed.

Study selection

Two independent reviewers (PDMA and AFS) nominated the articles that initially met the search criteria, based on their titles. After agreement on selected articles, the same 2 examiners read the abstracts independently. Once the agreement was again accessed, the articles were elected for full-text reading. The final inclusion of articles was done after discussion and verbal agreement between the 2 examiners. A third examiner (SCG) reviewed any disagreement between the examiners during all phases.

The selection of the articles was performed according to:

a. Type of study and patients: human randomized or non-randomized clinical trials (RCT/non-RCT) with at least 6 months of follow-up after periodontal treatment phase were included. The primary outcome of the original/primary papers should be microbiological data or clinical outcomes encompassing microbiological results as a secondary outcome. Studies were not included if: a) patients were not systemically healthy; or b) patients received implant prosthesis, surgical regenerative therapies, or were not previously treated to periodontal disease.
b. Type of interventions during PMP: studies with a PMP based on non-surgical interventions were included. The experimental interventions should be based on SPG or SPG+SBG interventions. When studies were based in antibiotics and/or supragingival chemical control, data concerning control groups, i.e. just mechanical intervention with or without placebo, were included in the analysis.
c. Type of outcomes: subgingival microbiological results were considered irrespective of the microbiological method used including both qualitative and/or quantitative analyses.

Quality assessment

Method of randomization, blinding of examiners, rate of patient losses during the follow-up, and the protocol and periodontal status information were evaluated to access the quality of the studies selected based on an adaptation of the Jadad scale.[18,19] Each item was scored: 0: not shown or absent information; 1: incomplete or unclear information, or 2: complete/adequate information. The achievement of a sum score of at least 4 was necessary to the study to be included (Appendix 2). A total score of 4 or 5 corresponded to an adequate quality, whereas a final score of 6 to 8 corresponded to a good quality.

Data extraction

Data were extracted considering: 1) the methodology of the studies and 2) the microbiological finds.

1. Study methodology:
   a. Study design: type of study, period of evaluation, experimental group included in the analysis, and the set out baseline moment of the PMP;
   b. Sample: size, gender, age, smoking habit, and initial periodontal diagnosis of the participants;
   c. Quality: data obtained from the quality assessment and respective final score;
   d. Treatment phase intervention: interventions employed before the PMP commencement;
   e. PMP intervention: type of intervention delivered at PMP appointments and recall frequency;
   f. Microbiological data: sampling and microbiological analysis methods.
2. Microbiological finds:
   a. Microbiological results: numerically expressed results for the target bacteria [*Aggregatibacter actinomycetemcomitans* (*A. actinomycetemcomitans*), *Porphyromonas gingivalis* (*P. gingivalis*), *Prevotella intermedia* (*P. intermedia*), *Treponema denticola (T. denticola), Tannerella forsythia* (*T. forsythia*), *Fusobacterium nucleatum* (*F. nucleatum*)], total bacterial counts and/or bacteria morph-types (mobile rods, spirochets, cocci).
   b. In the absence of numerically data, remarkable findings, *e.g.* important microbiological results as reported and /or stated by the authors, were collected. When applicable, previous and referenced studies were sought and analyzed to complete and/or clarify data from the included study.

Statistical analysis

Inter-examiner agreement for studies selection based on titles and abstracts was calculated using the Kappa coefficient.

As a consequence of the great discrepancy how results were presented by the authors, assessment of statistical heterogeneity or a meta-analysis could not be conducted.

Results

Searching results

The electronic searches resulted in 3,563 (MEDLINE) plus 879 (EMBASE) and the manual in 19 titles. After extracting duplicate citations, 2,250 potential articles remained to be screened (dated from 1968 on). From these, 148 after title and 39 after abstract analysis were considered to full-text evaluation. This evaluation resulted in the inclusion of 19 articles (Figure 1; Appendix 3). Kappa values for inter-examiner agreement were 0.71 and 0.79 for titles and abstract analyses, respectively.

Table 1 shows the results of the quality assessment of the included studies. All studies presented, at least, adequate quality (Appendix 2).

Among the included studies, none reported microbiological outcomes from direct comparisons between SPG versus SPG+SBG interventions. Five studies reported only SPG results, while 14 studies reported SPG+SBG. The characteristics of the included studies are shown in Table 1.

SPG results

Table 2 reports the microbiological data regarding the 5 SPG studies. The results from two studies[20,21] were interpreted by visual analysis from graphics and in accordance with the author's conclusions/statements.

Conventional Polimerase Chain Reaction (PCR): Chondros et al.[20] showed stability or even a decrease in the mean levels of *T. forsythia*, *A. actinomycetemcomitans*, *P. gingivalis*, *T. denticola* and *F. nucleatum* between 3 and 6 months of PMP, while P. intermedia presented a slight (non significant) increase.

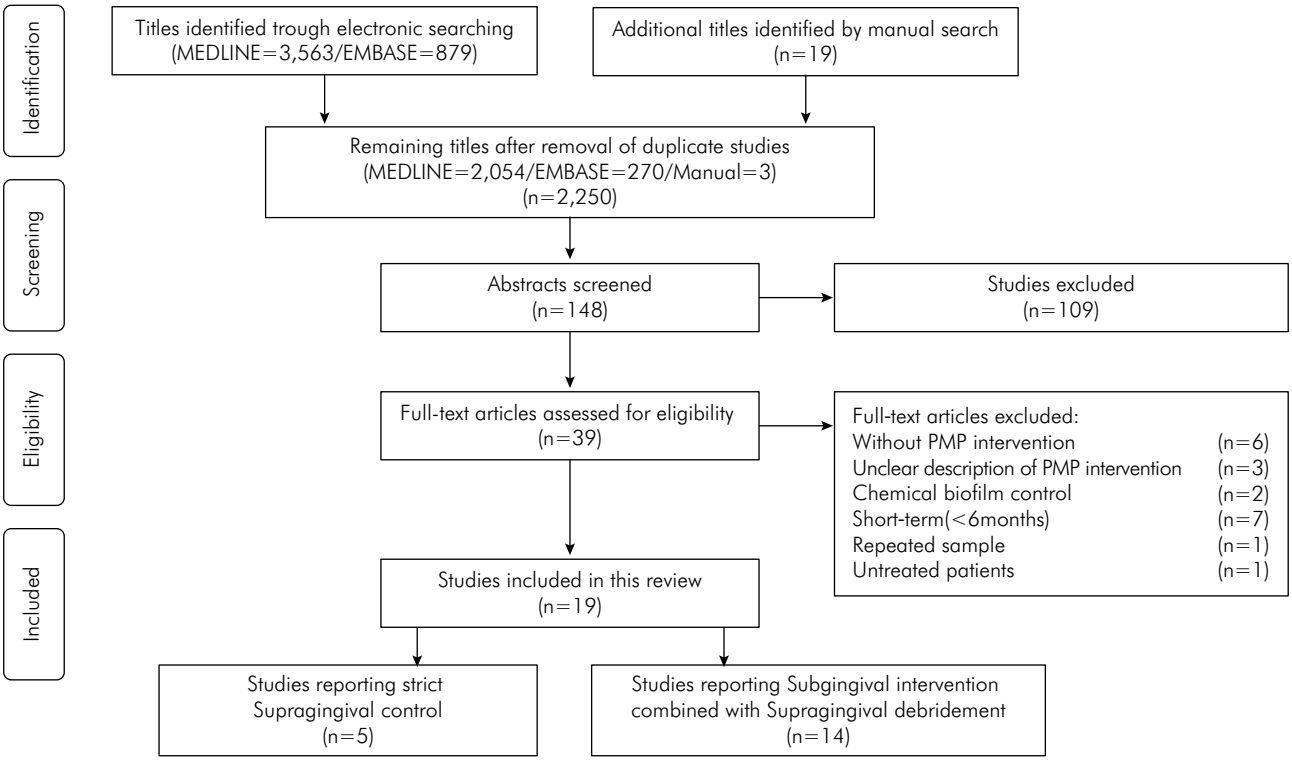

Figure 1. Flowchart of the studies selection.

Checkerboard DNA-DNA hybridization: Colombo et al.[21] showed that three months past the PMP beginning, the levels of all target species were maintained as stable, although the prevalence of *A. actinomycetemcomitans* and *F. nucleatum* experienced an increase.

Culture: Quirynem et al.[22] reported significant reductions in detection frequencies for *P. gingivalis* and *P. intermedia* in the first months of PMP, followed by stability over time. Rosling et al.[23] observed a small decrease on the total viable counts (TVC) between PMP baseline (15×10^6) and PMP-36-month (12×10^6) examination. Nevertheless, there was a significant reduction in the number of patients positive for *P. gingivalis* and *A. actinomycetemcomitans*, and a stability of those values for *P. intermedia*. Finally, in the study by Westfelt et al.[24] the mean TVC (10^6) were reduced between PMP baseline ($20.1 \pm 22 \times 10^6$) and 36-month ($5.4 \pm 11.9 \times 10^6$) examinations, and a decrease in the mean percentage of *P. gingivalis* (14.7 ± 25.4 to 0.4 ± 0.3); and *P. intermedia* (9.6 ± 11.6 to 3.5 ± 5.0) was also observed. Moreover, the number of patients positive for *A. actinomycetemcomitans* and *P. intermedia* was relatively stable over time, whereas a decrease was observed for *P. gingivalis* (4 patients to 1).

SPG + SBG results

The microbiological results of the 14 SPG+SBG studies are also presented in Table 2. Among these studies, only four reported the microbiological outcomes by numeric results on tables.[25,26,27,28]

Real-time PCR: Kolbe et al.[27] showed a non-significant trend to decrease the quantities (amounts: $\log_{10} \pm$ SEM) of *P. gingivalis* (1.6 ± 1.4 to 1.0 ± 1.4) and *T. forsythia* (4.9 ± 3.6 to 4.7 ± 3.8) from PMP baseline to 6 months, respectively, and stability for *A. actinomycetemcomitans* (2.7 ± 3.1 to 2.7 ± 2.5). There were no differences over time in the percentage of sites harboring *T. forsythia* and *A. actinomycetemcomitans*, whereas a significant decrease in the frequency of *P. gingivalis* was observed. In the same way, Müller et al.[28] reported an overall stability on microbiological outcomes since the number of positive sites with counts at > 1,000 and > 100,000 cells/ml were not significantly different before and after 12 months of PMP, for any of the target microorganisms.

Table 1. Characteristics of the included studies.

Study	Quality	Study design	Sample	Baseline intervention	PMP intervention	Microbiological data
			SPG studies			
Chondros et al. 2009[20]	Good	RCT; 6 mo; C group PMP: 3 mo on	12 individuals (7 females, 3 smokers, mean age 50.6 ± 9.2 years) enrolled in PMP. Chronic periodontitis (previously treated)	SRP plus Supra cleaning (sites PPD ≥ 4 mm)	Supra cleaning plus OHI at 3 mo interval	#50 paper point (20'); Deepest site per quadrant; PCR (micro-IDent® kit)
Colombo et al. 2005[21]	Adequate	Single-arm CT; 9 mo; Single group PMP: 3 mo on	25 individuals (15 females, 5 smokers, mean age 43±5 years). Untreated chronic periodontitis	Full-mouth SRP (4 to 6 weekly sessions) plus OHI	Supra prophylaxis plus OHI at 3 mo interval	Gracey curettes; 10 deepest sites (PPD ≥ 5 mm); Checkerboard DNA-DNA hybridization
Quirynem et al. 2005[22]	Good	RCT; 6 mo; C group PMP: 3 mo on	16 individuals. Moderate-to-severe periodontitis	One-stage full-mouth disinfection (SRP and rinsing with CHX gel) plus OHI	Polishing of all teeth plus OHI at 1-, 3- and 6 mo	4 medium paper points (10'); Single- and multi-rooted teeth in the maxillary right quadrant (3 deepest approximal sites/tooth type); Culture
Rosling et al. 1997[23]	Adequate	RCT; 36 mo; C group PMP: day 0 on	20 individuals enrolled in PMP. Advanced periodontal disease (previously treated)	No professional subgingival therapy	OHI at 3 mo interval	2 medium paper points (30'); Deepest site per quadrant; Culture
Westfelt et al. 1998[24]	Adequate	Split-mouth CT; 36 mo; C group PMP: day 0 on	12 individuals (9 smokers, 40–65 years). Advanced periodontal disease	Supra scaling plus OHI (every 2 weeks during 3mo); SRP in C group	Supra scaling plus OHI at 3 mo interval	2 paper points (15'); Deepest site per jaw quadrant (PPD > 5 mm); Culture
			SPG+SBG studies			
Bogren et al. 2008[29]	Good	RCT; 36 mo; C group PMP: 3 mo on	65 individuals (58 females, 31 smokers, mean age 60 years) enrolled in PMP. Moderate/ advanced chronic periodontitis (previously treated)	Supra- and subgingival instrumentation plus OHI	Subgingival debridement (BOP+, PPD ≥ 5 mm sites) plus supra polishing and OHI at 6 mo interval	Curettes; All mesial sites; Checkerboard DNA-DNA hybridization

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Cortelli et al. 2008[25]	Good	RCT; 24 mo; C group PMP: 3 to 12 mo	15 individuals (mean age 46.5 ± 12.2 years). Advanced chronic periodontitis	Full-mouth supra control plus SRP and application of placebo polymer (sites PPD≥6mm)	Regular supportive periodontal therapy plus placebo polymer application (sites PPD ≥ 6mm) at 3 mo interval	Paper points (10'); 2 non-molar homologous teeth (PPD ≥ 6 mm, BOP+, CAL+); PCR
Cugini et al. 2000[29]	Good	Single-arm CT; 12 mo; Single group PMP: 3 mo on	32 individuals (20 females, mean age 48 ± 11 years). Chronic periodontitis	SRP (one session per quadrant)	Full-mouth periodontal maintenance scaling plus OHI at 3 mo interval	Gracey curettes; All mesio-buccal sites; Checkerboard DNA-DNA hybridization
Ehmke et al. 2005[31]	Good	RCT; 24mo; C group PMP: 3mo on	17 individuals (8 female, mean age 53.2 ± 9.9 years). Moderate-to-severe chronic periodontitis (subgingival infection by *Aa* and *Pg*)	Supra- and subgingival scaling (per quadrant) plus OHI	Full-mouth supra debridement (3, 6, 9, 12, 18 and 24 mo) plus SRP at 12- and 18mo appointments (sites with CAL ≥ 2 mm compared to baseline)	Sterile curettes; Deepest site per quadrant (PPD ≥ 6 mm); PCR and PCR multiplex
Gunsolley et al. 1994[26]	Good	Single-arm CT; 12mo; Single group PMP: 3 mo on	13 individuals (up to 35 years old). Generalized severe aggressive periodontitis (previously treated)	SRP plus surgical treatment with open flap debridement (after 3mo)	Prophylaxis and OHI plus SRP at 3 mo interval	3 fine papers points (10'); 8 sites (PPD > 5 mm; CAL≥ 4 mm); Immunofluorescence
Haffajee et al. 2001[32]	Good	RCT; 6 mo; Both groups PMP: 3 mo on	48 individuals (C: manual toothbrush, n = 25, 42% females, mean age 47 ± 2 years, and T: powered toothbrush, n = 22, 41% females, mean age 49 ± 2 years, groups) enrolled in PMP. Chronic periodontitis (previously treated)	Full-mouth SRP (1 session) plus OHI	Maintenance scaling (SRP) and OHI at at 3 mo interval	Gracey curettes; All mesio-buccal sites; Checkerboard DNA-DNA hybridization
Kolbe et al. 2014[27]	Good	Split-mouth CT; 6 mo; C group PMP: day 0 on	22 individuals (12 females, non-smokers, mean age 48.52 ± 11.71 years). Chronic periodontitis	Supra control and full-mouth SRP (1 session)	Supra prophylaxis and OHI (every 15-days during 1mo and monthly until 6 mo) plus SRP (PPD > 5mm sites) at 3 mo appointment	1 paper point (30'); One site (PPD>5 mm and BOP+); Real-time PCR
Krohn-Dale et al. 2012[33]	Good	Split-mouth CT; 12 mo; Both groups PMP: 6mo on	15 individuals (3 females, all smokers, mean age 57.7 years) enrolled in PMP. Chronic periodontitis (previously treated)	Er:YAG laser or ultrassonic scaler and curette instrumentation (BOP+ residual pockets) plus full mouth supra cleaning and OHI	SRP plus supra cleaning and OHI at 3 mo interval	4 paper points (30'); 2 sites (BOP+ deepest non-adjacent pockets) per jaw-quadrant per group; Checkerboard DNA-DNA hybridization

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Listgarten et al. 1989[34]	Adequate	RCT; 48mo; C group PMP: day 0 on	47 individuals (29 females, mean age 55.4 ± 1.5 years) enrolled in PMP. Chronic periodontitis (previously treated)	Periodontal prophylaxis by a dental hygienist	Periodontal prophylaxis by a dental hygienist at 3 mo interval	6 deepest sites; Differential dark field microscopic
McColl et al. 2006[35]	Good	RCT; 12mo; C group PMP: day 0 on	19 individuals (12 females, 5 smokers, mean age 45 ± 7 years). Moderate-to-advanced chronic periodontitis (previously treated)	Piezo-ceramic scaler and curettes subgingival debridement (sites PPD≥5 mm plus BOP+)	Subgingival debridement (sites PPD≥5 mm plus BOP+) plus OHI at 3mo interval	2 paper points; 4 deepest sites; Checkerboard DNA-DNA hybridization
Müller et al. 2014[28]	Good	Split-mouth CT; 12 mo; Control group PMP: 3 mo on	50 individuals (29 females, 19 smokers, mean age 58.5 years) enrolled in PMP. Periodontal disease (previously treated)	Ultrassonic SRP in all PPD > 4mm sites of 2 control quadrants plus OHI	Subgingival debridement in all PPD>4 sites plus OHI at 3mo interval	1 paper point (10'); deepest site; Real-time PCR (Sybr Green)
Murray et al. 1989[36]	Adequate	Matched CT; 12 mo; Both groups PMP: day 0 on	40 individuals (C: manual toothbrush, n=20, 14 females, mean age 29.2 years, and T: powered toothbrush, n=20, 14% females, mean age 34.3 years, groups) enrolled in PMP. Moderate-to-advanced periodontitis (previously treated)	1 hour-long session of subgingival debridement plus OHI	OHI (at every 3 mo) and subgingival debridement at 6- and 12 mo appointments	3 fine paper points (10'); Mesio-buccal (maxillary right 1st molar) and disto-buccal (lower left central incisor) sites; Culture and dark field microscopic
Teles et al. 2008[37]	Adequate	Non-RCT; 36 mo; Maintenance group PMP: day 0 on	62 individuals (30 females, 8 smokers, mean age 57±10 years) enrolled in a previous RCT with different PMP. Chronic periodontitis (previously treated)	Supra- and subgingival instrumentation plus polishing and OHI	Supra- and subgingival instrumentation plus polishing and OHI at 3-6mo interval	Gracey curettes; All mesio-buccal sites; Checkerboard DNA-DNA hybridization
Ximenez-Fyvie et al. 2000[38]	Good	Single-arm CT; 12 mo; Single group PMP: 3 mo on	18 individuals (7 females, mean age 52 ± 12 years) enrolled in PMP. Chronic periodontitis (previously treated)	SRP (4 sessions) and supra plaque removal and OHI oral (weekly in the first 3mo)	Subgingival maintenance scaling at 3 mo interval	Gracey curettes; All mesio-buccal sites; Checkerboard DNA-DNA hybridization

Aa: *Aggregatibacter actinomycetemcomitans;* BOP: bleeding on probing; C group: control group; CAL: clinical attachment loss; CHX: chlorhexidine; CT: clinical trial; Mo: month; non-RCT: non-randomized clinical trial; OHI: oral hygiene instructions; PCR: polymerase chain reaction; *Pg*: *Porphyromonas gingivalis;* PMP: periodontal maintenance phase; PPD: periodontal probing depth; RCT: randomized clinical trial; SRP: scaling and root planing; SPG: supragingival control; SPG+SBG: subgingival scaling and root planing intervention performed combined with supragingival debridement; T group: test group.

Table 2. Microbiological outcomes of the included studies.

Study	Microbiological results or remarkable findings as stated by the authors of the studies
	SPG studies
Chondros et al. 2009[20]	Data expressed as mean bacterial levels. No information was reported regarding intra-group comparisons in the control group. "both treatments resulted in significant reductions in [mean level of] *T. denticola, F. nucleatum,* (…). The only differences between the two treatments were statistically significant reductions of (…) *E. corrodens* and *Capnocytophaga sp.* at 6mo after treatment with PDT."
Colombo et al. 2005[21]	Data expressed as mean detection frequency (%) and mean levels count (x 10^5 cells). "Significant reductions in the prevalence and levels were observed for *P. gingivalis*, *T. forsythia* and *A. actinomycetemcomitans*. Although this last species reduced significantly in counts over time, its frequency increased to baseline values at 9 mo post-therapy." "Species of *Prevotella* showed a modest decrease in prevalence, however, their levels were markedly reduced." "the most striking changes in prevalence and levels of the majority of the microorganisms occurred during the first 3mo after SRP, although several species still presented lower levels at 9mo when compared with baseline values." "these results indicate that periodic maintenance visits are needed to keep the pathogenic species at lowered levels."
Quirynem et al. 2005[22]	Data expressed as detection frequency [n = 16], and mean changes in total number of CFU/ml for aerobic and anaerobic species. SR: single-rooted teeth; MR: multi-rooted teeth. *P. gingivalis*: 3 mo: SR: 3, MR: 3; 6 mo: SR: 2, MR: 3; *P. intermedia*: 3 mo: SR: 11, MR: 13; 6 mo: SR: 10, MR: 13. "The number of aerobic as well as anaerobic species around SR (…) remained nearly unchanged for the placebo group (small treatment effect with a 0.3 log reduction)." "For the MR the changes were comparable with similar intra- as well as inter-product variations."
Rosling et al. 1997[23]	Data expressed as total viable counts (TVC x 10^6): Day 0: 15 x 10^6; 36 mo: 12 x 10^6. Data expressed as mean % of bacteria in positive samples (number positive patients) [n = 20]: *A. actinomycetemcomitans*: Day 0: 0.1(5); 36 mo: 0.1(1); *P. gingivalis*: Day 0: 7(9); 36 mo: 2(4); *P. intermedia*: Day 0: 4(19); 36 mo: 2(16).
Westfelt et al. 1998[24]	Data expressed as mean total viable counts (TVC x 10^6): Day 0: 20.1 ± 22.0; 36mo: 5.4 ± 11.9. Data expressed as mean % of bacterial species (number positive patients) [n = 12]: *A. actinomycetemcomitans*: Day 0: 0.6 ± 1.2 (5); 36 mo: 4.0 ± 8.6 (4); *P. gingivalis*: Day 0: 14.7 ± 25.4 (4); 36mo: 0.4 ± 0.3 (1); *P. intermedia*: Day 0: 9.6 ± 11.6 (12); 36 mo: 3.5 ± 5.0 (12).
	SPG + SBG studies
Bogren et al. 2008[29]	Data expressed as mean counts (x 10^5). Results just regarding sites PPD ≥ 5 mm. C: control group; T: test group. "Mean counts of 13 of 40 and 8 of 40 target species changed significantly over time in the T and C groups, respectively. In particular, species in the green complex showed significant reductions over time in C group, whereas species in the green and orange complexes were significantly reduced over time in the T group."
Cortelli et al. 2008[25]	Data expressed as mean bacteria values (frequencies of bacteria) [n = 15]. *A. actinomycetemcomitans*: 3 mo: 3.70(2); 6 mo: 3.30(0); 9 mo: 3.30(0); 12 mo: 3.30(0); *P. gingivalis*: 3 mo: 3.67(4); 6 mo: 3.27(2); 9 mo: 3.27(2); 12 mo: 3.27(2); *P. intermedia*: 3 mo: 4.20(5); 6 mo: 3.40(1); 9 mo: 3.20(0); 12 mo: 3.20(0); *F. nucleatum*: 3 mo: 3.23(3); 6 mo: 3.23(3); 9 mo: 3.43(4); 12 mo: 3.43(4).

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Cugini et al. 2000[30]	Data expressed as prevalence and levels, and mean % of DNA probe counts. "*P. gingivalis*, *T. forsythia* and *T.denticola* decreased in prevalence and levels up to the 6mo visit and remained at these lower levels at 9- and 12mo". "levels and prevalence *T. forsythia*, *P. gingivalis* decreased significantly". "proportion of the total DNA probe count that *T. forsythia*, *P. gingivalis* comprised was significantly decreased at 12mo". "[*T. forsythia* and *P. gingivalis*] declined in prevalence until 6mo and showed a slight increase at 9- and 12mo post therapy whereas proportions continued to decrease. The decline in proportion of these species paralleled the decrease in PPD." "most profound reduction occurred during the first 3mo post SRP although these species were still reduced significantly at 12mo when compared with pre-treatment levels. Thus, maintenance scaling appeared to be important in maintaining the initial post therapy decreases in selected species for prolonged periods of time."
Ehmke et al. 2005[31]	Data expressed as % of patients colonized. C: control group; T: test group "both therapies had only a limited influence on the prevalence of the majority of assessed periodontal pathogens. With the exception of *A. actinomycetemcomitans* no long-term eradication of pathogens was registered over the entire study period." "No additional differences in detection frequencies of *P. gingivalis* were found between T and C group patients over the study period." "No significant differences were found between T and C group concerning the prevalence of *T. forsythia*, *Treponema ssp.*, and *P. intermedia*." "In 5 T-group patients and 1 C-group patient, *A. actinomycetemcomitans* was suppressed over the 24mo study period. In the remaining patients, *A. actinomycetemcomitans* was temporarily suppressed or persisted."
Gunsolley et al. 1994[26]	Data expressed as mean levels (mean proportion of positive sites). *A. actinomycetemcomitans*: 3mo: 0.18±0.06 (0.12±0.03); 6mo: 0.27±0.14 (0.08±0.03); 9mo: 0.07±0.04 (0.05±0.02); 12mo: 0.14±0.06 (0.07±0.02). *P. gingivalis:* 3mo: 0.08±0.06 (0.03±0.17); 6mo: 0.09±0.05 (0.04±0.02); 9mo: 0.07±0.03 (0.05±0.02); 12mo: 0.68±0.20 (0.14±0.03).
Haffajee et al. 2001[32]	Data expressed as mean total counts ($x10^5$); mean number of positive patients (%). M: manual brushing; P: powered brushing. "There was a significant decrease in total counts for (...) subgingival plaque samples in the subjects using the manual brush and a significant decrease in subgingival counts for the P group. The majority of subjects in both groups showed a decrease in total counts from baseline to 6 mo." "All taxa examined were reduced in prevalence for both brushing groups. Only *A. actinomycetemcomitans* increased in prevalence in both groups." "prevalence of the red complex species, *T. forsythia*, *P. gingivalis*, and *T. denticola* was markedly decreased for most subjects." "The major finding was the effect of supragingival plaque removal on the composition of the subgingival microbiota."
Kolbe et al. 2014[27]	Data expressed as amount (log_{10} ± SEM) and percentage of positive sites: *A. actinomycetemcomitans*: Day 0: 2.7 ± 3.1 (47.1%); 3 mo: 1.6 ± 2.1 (47.1%); 6 mo: 2.7 ± 2.5 (64.7%); *P. gingivalis*: Day 0: 1.6 ± 1.4 (58.8%); 3 mo: 1.5 ± 1.4 (52.9%); 6 mo: 1.0 ± 1.4 (35.5%); *T. forsythia*: Day 0: 4.9 ± 3.6 (68.18%); 3 mo: 5.3 ± 2.8 (63.63%); 6 mo: 4.7 ± 3.8 (63.63%).
Krohn-Dale et al. 2012[33]	Data expressed as total bacteria scores and number of positive patients. C: control group; T: test group. "Total bacterial scores showed an overall significant difference by time for the C group (p = 0.002) and a borderline difference for the T group (p = 0.05). The difference between groups was not significant. Comparable values of bacterial counts were recorded at different time points for the two treatments." "In the C group, a significant reduction in bacterial counts was observed from baseline to 6 mo (p = 0.008) and to 12 mo (p = 0.003), whereas the reduction between 6 mo and 12 mo was not significant. The reduction in bacterial counts for the T group from baseline to 6- and 12 mo was close to significant, whereas the reduction between 6- and 12 mo was not significant." "For both treatments, the prevalence of *P. gingivalis* decreased significantly from baseline to 6mo (control: p = 0.016; test: p = 0.039), and within the T group, a significant reduction was observed from baseline to 12 mo (p = 0.016). At 12 mo, *P. gingivalis* was totally eradicated in the C group, whereas one patient in the T group harbored *P. gingivalis*." "prevalence of *T. forsythia* decreased significantly from baseline to 6 mo within the T group (p = 0.021) and the pathogen was not detected at 12 mo. In the C group, the reduction from baseline to 6- and 12 mo was not significant." "significant reduction in prevalence of *A. actinomycetemcomitans* from baseline to 6 mo was only achieved for the C group (p = 0.008). From 6 to 12 mo, a rebound was observed for both treatments."

Continuation

Listgarten et al. 1989[34]	Data expressed as mean proportion of bacterial morphotypes. C: control group; T: test group. "proportions of coccoid cells were not significantly different between the C and T groups. Proportions were highest during the first 6 mo, dropping sharply at the 1-year examination. (...) the proportions increased slightly with time, but never reached baseline levels." "% of motile rods means did not differ significantly between groups, nor did they differ between examinations." "mean proportions of spirochetes throughout the study was similar for the C and T group. The proportions decreased from baseline through the first 18mo. After stabilizing for the next 18mo, they began to increase again." "percentages of "other" bacterial morphotypes for both treatment groups increased from the baseline and 6mo examinations to the 1-year and subsequent examinations. There were no differences between the proportions of other bacteria for the C and T groups." "patients in the T group fared as well as the C patients both clinically and microbiologically."
McColl et al. 2006[35]	Data expressed as prevalence (%) of individuals/ sites with counts > 10^5. D-group: control group. "There was a general pattern of increasing prevalence of bacterial pathogens with increasing time in both groups. From 6 mo on, there were significantly more patients in the D-group with high counts of *P. gingivalis* and *T. forsythia* than in the M-group. No difference was found for *A. actinomycetemcomitans* and *P. intermedia*." "At 12 mo, 68% [of individuals] in the D-group had one or more sites with at least two species of the Red Complex at counts > 10^5. The corresponding percentage of the sites amounted 35.9%, respectively." "prevalence of *P. gingivalis*, *T. forsythia*, *A. actinomycetemcomitans*, *P. intermedia* remained at levels ≤ 10^5 in the majority of patients and sites in both groups."
Müller et al. 2014[28]	Data expressed as numbers of positive sites with counts > 1000 and > 100,000 cells/ml (respectively) [n = 50]. Day 0: *A. actinomycetemcomitans:* 7 and 0; *T. forsythia:* 39 and 15; *P. gingivalis:* 37 and 12; *T. denticola:* 42 and 27; *P. intermedia:* 14 and 6; 12mo: *A. actinomycetemcomitans:* 10 and 2; *T. forsythia*: 35 and 19; *P. gingivalis:* 34 and 10; *T. denticola:* 38 and 17; *P. intermedia:* 11 and 4. "The detection frequencies of the studied microorganisms at >1,000 and >100,000 cells/ml were not significantly different before and after 12 months. However, at the final examination the frequency of sites with counts of *A. actinomycetemcomitans* >1000 cells/ml was lower in the test compared to the control group, and no sample contained >100,000 cells/ml, compared to two in the control group."
Murray et al. 1989[36]	Data expressed as log of the total CFU. C: conventional oral hygiene group; R: rotary tooth cleaner group; Obligate anaerobic bacteria: Day 0: C: 63.9% - R: 61.8%; 12mo: C: 30.2% - R: 33.1%. "for this patient group, microbiologic status paraleled clinical status for the 12-month study period."
Teles et al. 2008[37]	Data expressed as mean total counts (x 10^5): Day 0: 37.7 x 10^5; 36 mo: 20.9 x 10^5. "Both clinical groups showed statistically significant reductions in the total mass of subgingival biofilm as measured by the total DNA probe counts"
Ximenez-Fyvie et al. 2000[38]	Data expressed as mean total and individual species counts (x 10^5). "Mean total counts and mean counts of individual species (34/40) decreased after completion of the professional cleaning phase and continued to decrease even though the subjects returned to self-performed plaque control after the 3mo monitoring visit." "The major effect was seen at 3mo, immediately after completion of the professional cleaning phase, for *P. gingivalis* and *T. forsythia*. Mean counts of *A. actinomycetemcomitans* were decreased at 3mo but continued to decline between 3- and 6mo." "reduction in mean counts observed was due in part to a decrease in prevalence (% of sites colonized), but more to a reduction in the % of sites exhibiting high counts of the test species. 5 species were significantly decreased. *P. gingivalis* was found not only at significantly fewer sites but in significantly lower numbers." "Therapy employed was able to establish a host compatible microbiota for a prolonged period of time."

A. actinomycetemcomitans: Aggregatibacter actinomycetemcomitans; P. gingivalis: Porphyromonas gingivalis; P. intermedia: Prevotella intermedia; T. denticola: Treponema denticola; T. forsythia: Tannerella forsythia; F. nucleatum: Fusobacterium nucleatum; CFU: colony-forming unit; CHX: chlorhexidine; mo: months; PDT: photodynamic therapy; PPD: periodontal probing depth; SRP: scaling and root planing; SPG: supragingival control; SPG+SBG: subgingival scaling and root planing intervention performed combined with supragingival debridement.

Conventional PCR: Cortelli et al.[25] observed no significant differences between PMP time-points in the presence of target pathogens. Irrespective of theses observations, reductions on mean bacteria values for *A. actinomycetemcomitans* (3.70 to 3.30) and *P. intermedia* (4.20 to 3.20), between 3 and 12 months of PMP, respectively, were significant. Ehmke et al.[31] showed that the results related to the prevalence of *A. actinomycetemcomitans* and *P. gingivalis* presented a general stability along PMP. However, an increase in the frequency of *P. intermedia* and *T. forsythia* could be observed. Interestingly, these values were somewhat similar to those observed at pre-treatment examination.

Checkerboard DNA-DNA hybridization: studies performed by Bogren et al.[29], Haffajee et al.[32], Teles et al.[37] and Ximenez-Fyvie et al.[38] showed that the mean levels/counts (x10^5; SEM) of the target species remained constant[29] or even decreased[32,37,38] during PMP. The results from Cugini et al.[30] showed an overall stability in prevalence and counts of all target bacteria, especially for *T. forsythia* and *P. gingivalis*, with some fluctuations between study time-points, although the increases never reached the pre-treatment values. Krohn-Dale et al.[33], in turn, observed a significant decrease in the total bacterial scores and number of positive patients for the target bacteria between PMP-day 0 and 6-month examination, followed by stability or even a light decreasing until the end of the study. On the other hand, there was a rebound for A. actinomycetemcomitans at the PMP-12-month exam. In opposition, McColl et al.[35] showed a general pattern of increase in the number of individuals or sites (counts > 10^5) positive to the target bacteria species over PMP, with the final numbers always greater than the pre-treatment values.

Immunofluorescence: Gunsolley et al.[26] showed a light and continuing decrease in the mean levels of *P. gingivalis* (0.08 ± 0.06 to 0.07 ± 0.03) and *A. actinomycetemcomitans* (0.18 ± 0.06 to 0.07 ± 0.04) between PMP-3 to 9-months examinations, respectively. At the end of the study (12 months), the levels of *P. gingivalis* (0.68 ± 0.20) increased, *A. actinomycetemcomitans* remaining stable (0.14 ± 0.06).

Dark field microscopy: Listgarten et al.[34] reported that the mean proportions of different bacterial morph-types were maintained stable during PMP. However, the mean values for the so-called "other bacteria morph-types" increased from PMP-day 0 and 6-month examinations to 1-year and subsequent examinations. Finally, Murray et al.[36] showed a significant shift in the microbiota for the experimental group that experienced high percentage of obligate anaerobes, motile rods, and spirochetes at the beginning of PMP. After 12 months, the microbiota showed similarity to that described as associated to oral health status. *F. nucleatum* (log) total colony-forming unit (CFU) values were relatively unchanged from day 0 to 6-months, but had significantly decrease at 12-months.

Discussion

The present study sought to investigate subgingival microbiological outcomes during the PMP when supragingival biofilm control alone or its combination with the subgingival one were delivered. Although no randomized clinical trial aiming to answer this question was available, the results from studies with SPG or SPG + SBG experimental groups showed that both interventions were able to propitiate stability on the microbiological results over time.

There was an expressive variation in PMP baseline definition in the studies once a clear distinction between treatment end and PMP commencement phase were absent in most of them. Because of that, the 90th day after therapy was considered the PMP baseline to data collection.[1,39,40] If the 90th day data was not available, the nearest exam was considered. This was applied to 3 out of 5 SPG studies, and in 8 out of 13 SPG+SBG studies. Besides, most of the studies included presented short-term evaluations of no longer than 12 months of PMP. It is stated that this is a short period to observe great changes in periodontal status over time, and that the major challenges for a breakdown or recurrence of the disease occur later in the maintenance phase.[2,8,37,41,42] Herein, microbiological findings in studies with more than 12 months showed in general stability along subsequent years.[23,24,29,34,37]

The methodology for microbiological identification performed by most studies were qualitative or semi-quantitative analyses, when it is accepted that molecular identification and absolute quantification are the preferred techniques.[43,44] Irrespective, the

overall results showed constancy of the subgingival microbiological outcomes during PMP related to both interventions, even thought changes in bacteria numbers could be observed within experimental time-points. These fluctuations were particularly observed in the SPG+SBG studies reporting the percentage of positive patients/sites (*e.g.*, prevalence) by the use of Checkerboard DNA-DNA hybridization[30,33,35] or a non-quantitative method (dark field microscopy[34] and conventional PCR[31]). Interestingly some of the fluctuations observed indicated eradication of some bacterial type. However, as it is known that the eradication of oral pathogens residents is not possible and even an undesirable goal,[14,16] this so called eradication should be interpreted with caution. The absence or disappearance of bacterial specie might be related to limitations of the methods.[43,44]

The main limitation of the preset study is that it was not possible to perform a statistical analysis. Results reported only graphically and differences between studies methods, such as different periods of evaluation and the microbiological method applied, made the meta-analyses impossible. However, the pattern of microbiological response was very consistent between studies, irrespective of the applied therapy: SPG or SPG + SBG. In addition, in accordance with the literature,[12,13,14] it was observed a close relationship between these microbiological and the clinical outcomes reported, somewhat reinforcing the similarity of response between the PMP investigated protocols. Notwithstanding, similar findings have already been reported by Heasman et al.[10] in a systematic review of clinical data from different studies others than those included in the present review.

Conclusion

The importance of the present results relies on the fact that microbiological outcomes during PMP are, in part, considered cause of failure of the periodontal stability over time. Consequently, this assumption reinforces the need to access the subgingival area as to reduce the chances to lose attachment levels along time. However, it seems that both SPG and SPG + SBG regimens during PMP determined stability in the microbiological results along time. Nevertheless, new studies comparing this outcome are still needed aiming to improve the quality of the future researches on this field.

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Appendix 1 - A. Electronic search strategy for MEDLINE database and number of found studies.

Strategie	MesH terms and combinations	Nº studies
#1	Randomized clinical Trial AND supportive periodontal care	23
#2	Randomized clinical trial AND periodontal maintenance	185
#3	Randomized clinical trial AND supportive periodontal care AND microbiology	9
#4	Randomized clinical trial AND periodontal maintenance AND microbiology	40
#5	Randomized controlled trial AND supportive periodontal care	25
#6	Randomized controlled trial AND periodontal maintenance	183
#7	Randomized controlled trial AND supportive periodontal care AND microbiology	9
#8	Randomized controlled trial AND periodontal maintenance AND microbiology	40
#9	Clinical trial AND supportive periodontal care	31
#10	Clinical trial AND periodontal maintenance	256
#11	Clinical trial AND supportive periodontal care AND microbiology	11
#12	Clinical trial AND periodontal maintenance AND microbiology	53
#13	Longitudinal study AND supportive periodontal care	19
#14	Longitudinal study AND periodontal maintenance	151
#15	Longitudinal study AND supportive periodontal care AND microbiology	2
#16	Longitudinal study AND periodontal maintenance AND microbiology	29
#17	Prospective study AND supportive periodontal care	11
#18	Prospective study AND periodontal maintenance	94
#19	Prospective study AND supportive periodontal care AND microbiology	3
#20	Prospective study AND periodontal maintenance AND microbiology	11
#21	Supportive periodontal care AND microbiology	28
#22	Periodontal maintenance AND microbiology	217
#23	Supportive periodontal care	180
#24	Periodontal maintenance	1,953
Total		3,563

Appendix 1 - B. Electronic search strategy for EMBASE database and number of found studies.

Strategie	Key-words and combinations	N° studies
#1	randomized AND clinical AND trial AND supportive AND periodontal AND care AND [embase]/lim	7
#2	randomized AND clinical AND trial AND periodontal AND maintenance AND [embase]/lim	31
#3	randomized AND clinical AND trial AND supportive AND periodontal AND care AND microbiology AND [embase]/lim	1
#4	randomized AND clinical AND trial AND periodontal AND maintenance AND microbiology AND [embase]/lim	5
#5	randomized AND controlled AND trial AND supportive AND periodontal AND care AND [embase]/lim	7
#6	randomized AND controlled AND trial AND periodontal AND maintenance AND [embase]/lim	29
#7	randomized AND controlled AND trial AND supportive AND periodontal AND care AND microbiology AND [embase]/lim	1
#8	randomized AND controlled AND trial AND periodontal AND maintenance AND microbiology AND [embase]/lim	5
#9	clinical AND trial AND supportive AND periodontal AND care AND [embase]/lim	17
#10	clinical AND trial AND periodontal AND maintenance AND [embase]/lim	64
#11	clinical AND trial AND supportive AND periodontal AND care AND microbiology AND [embase]/lim	1
#12	clinical AND trial AND periodontal AND maintenance AND microbiology AND [embase]/lim	8
#13	longitudinal AND study AND supportive AND periodontal AND care AND [embase]/lim	1
#14	longitudinal AND study AND periodontal AND maintenance AND [embase]/lim	18
#15	longitudinal AND study AND supportive AND periodontal AND care AND microbiology AND [embase]/lim	0
#16	longitudinal AND study AND periodontal AND maintenance AND microbiology AND [embase]/lim	2
#17	prospective AND study AND supportive AND periodontal AND care AND [embase]/lim	6
#18	prospective AND study AND periodontal AND maintenance AND [embase]/lim	15
#19	prospective AND study AND supportive AND periodontal AND care AND microbiology AND [embase]/lim	3
#20	prospective AND study AND periodontal AND maintenance AND microbiology AND [embase]/lim	0
#21	supportive AND periodontal AND care AND microbiology AND [embase]/lim	7
#22	periodontal AND maintenance AND microbiology AND [embase]/lim	51
#23	supportive AND periodontal AND care AND [embase]/lim	84
#24	periodontal AND maintenance AND [embase]/lim	516
Total		879

Appendix 2. Quality assessment of the included studies.

Intervention	Study/design	Randomization	Blinding	Rate of loss	Protocol
			Randomized clinical trials		
SPG	Chondros et al. 2009[20]	2	2	2	2
	Quirynem et al. 2005[22]	2	2	2	2
	Rosling et al. 1997[23]	1	2	0	2
			Single-arm clinical trial		
	Colombo et al. 2005[21]	NA	NA	2	2
			Split-mouth clinical trial		
	Westfelt et al. 1998[24]	1	NA	1	2
			Randomized clinical trials		
SPG + SBG	Bogren et al. 2008[30]	2	2	2	2
	Cortelli et al. 2008[25]	1	2	2	2
	Ehmke et al. 2005[29]	1	2	2	2
	Haffajee et al. 2001[31]	1	2	2	2
	Listgarten et al. 1989[37]	1	0	2	1
	McColl et al. 2006[36]	2	2	2	2
			Non-randomized clinical trial		
	Teles et al. 2008[32]	NA	NA	2	2
			Single-arm clinical trials		
	Cugini et al. 2000[34]	NA	2	2	2
	Gunsolley et al. 1994[26]	NA	2	2	2
	Ximenez-Fyvie et al. 2000[33]	NA	2	2	2
			Matched clinical trial		
	Murray et al. 1989[38]	NA	2	2	1
			Split-mouth clinical trials		
	Kolbe et al. 2014[27]	2	2	2	2
	Krohn-Dale et al. 2012[35]	2	2	2	2
	Müller et al. 2014[28]	2	2	2	2

0: not shown/absent; 1: incomplete/unclear; 2: complete information; NA: not applicable. SPG: supragingival control; SPG + SBG: subgingival scaling and root planing intervention performed combined with supragingival debridement.

Appendix 3. Excluded full-text analyzed studies.

Authors and publication year of the study	Database source	Reason of exclusion
Byrne et al. 2009	E	Without PMP intervention
Carvalho et al. 2015	E	Unclear PMP intervention
Charalampakis et al. 2013	E	Without PMP intervention
Cosyn et al. 2013	E	Short-term
Escribano et al. 2010	E	Short-term
Giedrys-Leeper et al. 1985	E	Short-term
Guarnelli et al. 2010	E	Short-term
Haffajee et al. 1997	M	Repeated sample
Haffajee et al. 2009	E	Chemical biofilm control
Jansson et al. 2004	E	Short-term
Jolkovsky et al. 1990	E	Unclear PMP intervention
Listgarten et al. 1986	E	Short-term
Magnusson et al. 1984	E	Chemical biofilm control
Müller Campanile et al. 2015	E	Without PMP intervention
Petersilka et al. 2003	E	Unclear PMP intervention
Ratka-Krüger et al. 2012	E	Without PMP intervention
Riep et al. 1999	E	Short-term
Rudhart et al. 1998	E	Without PMP intervention
Smulow et al. 1983	M	Untreated patients
Wong et al. 1999	E	Without PMP intervention

E: electronic search; M: manual search;