Systematic Review
Periodontics

Systematic review of the effect of probiotics on experimental gingivitis in humans

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Abstract: Probiotic therapy is a viable alternative to chlorhexidine, a widely used antiseptic in dentistry that produces significant adverse effects. This systematic review aimed to analyze the effects of probiotics on experimental gingivitis in humans. Two independent reviewers conducted a comprehensive literature search until March 2019. Randomized clinical trials and controlled clinical trials were selected. Outcome data were extracted and critically analyzed. A total of five articles were included in the qualitative synthesis. No meta-analysis could be conducted due to the heterogeneity of the selected studies. The use of probiotics showed a slight improvement in clinical parameters. Changes in gingival crevicular fluid volume were lower in the presence of the probiotic than in the placebo group. All the studies showed that the immediate, positive effects of probiotics during the period of discontinued mechanical oral hygiene were due to the modulation of the host response, not the anti-plaque effect. Investigators should conduct randomized clinical trials to elucidate the mechanisms of probiotic action and develop improved delivery systems.

Keywords: Immunomodulation; Inflammation; Gingival Crevicular Fluid; Microbiota; Probiotics.

Declaration of Interests: The authors certify that they have no commercial or associative interest that represents a conflict of interest in connection with the manuscript.

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Introduction

The use of chemical agents in bacterial plaque control has been recommended for patients with greater susceptibility to gingivitis.[1] However, prolonged use of antiseptics may be associated with side effects such as tooth staining, taste alteration and mucosal desquamation.[2] Probiotics are live microorganisms which, when given in adequate amounts confer a health benefit on the host.[3] They have shown promising potential as an alternative to mouthwashes that can cause undesired effects.

Dental caries and periodontal disease are the most common infectious diseases in humans, with approximately 90% prevalence.[4] Recently, there has been an increasing interest in using probiotics to manage oral infections, and some controlled clinical studies have been conducted to elucidate the potential impact of probiotics on oral health.[5,6,7,8] According to a study, probiotic intervention in childhood reduced salivary mutans streptococci and decreased the risk of dental caries,[5] Krasse et al.[6] showed a significant decrease in gingival bleeding with the administration of probiotic *Lactobacillus*

reuteri. Oral administration of *Lactobacillus salivarius* WB21 reduced the levels of periodontopathic bacteria and improved periodontal status.[7] The adjunctive use of probiotic tablets effectively inhibited periodontal pathogens and reduced inflammation in patients with gingivitis.[8] However, the consumption of a probiotic milk beverage containing *Lactobacillus casei* strain Shirota showed no significant difference in experimental gingivitis between test and control groups.[9]

Although some studies with probiotics did not show improvements in the clinical parameters associated with gingivitis, they were able to demonstrate biomarker responses and modulate the activity of inflammatory cytokines, suggesting that host response could be regulated with the use of probiotics.[6,9,10,11]

Probiotics may be a suitable alternative to chlorhexidine, a widely used antiseptic in dentistry with potential side effects. Moreover, it has been predicted that the market for probiotic-containing supplements will grow from $35 billion to $48 billion by 2020.[12] This study aimed to systematically review randomized clinical trials on the effect of probiotics on experimental gingivitis.

Methodology

Protocol registration

This study was registered in PROSPERO (CRD42018116873). The Systematic Review followed PRISMA-P guidelines.[13] The SR methodology followed the recommendations of the Cochrane Handbook for Systematic Reviews of Interventions.[14] PRISMA checklist was used to ensure the quality and transparency of the study.[15] The PICOS strategy was used to construct a focused question.[16]

Focused question

What are the effects of probiotics on clinical and biomolecular signs of inflammation in human experimental gingivitis?

Clinical relevance

Probiotics may be a useful therapeutic alternative to facilitate post-operative healing, as opposed to antimicrobial products.

Search strategy

A systematic search without date or language restrictions was performed using electronic databases such as PubMed/MEDLINE, Cochrane Library (CENTRAL), Web of Science and Trip until March 2019. A literature search of the following journals was conducted to complement the electronic search: *Journal of Periodontology, Journal of Clinical Periodontology, Journal of Periodontal Research, The International Journal of Periodontics and Restorative Dentistry,* and *Clinical Oral Investigations*. A search of the Grey Literature Report[17] and OpenGrey databases[18] revealed unpublished studies (grey literature). Finally, the reference/bibliography lists of all full-text articles (cross-referencing) and the ClinicalTrials.gov database were searched.

Eligibility criteria based on PICOS strategy

The present SR included studies that analyzed the effect of probiotics on the outcomes of experimental gingivitis (Table 1).

Population: Human adults presenting experimental gingivitis.

Intervention: use of probiotic therapy.

Comparison: use of placebo.

Outcomes: the primary outcome was gingivitis identified and graded by bleeding on probing (BOP), plaque index (PI), and gingival index (GI). The secondary outcome was the inflammatory response determined by gingival crevicular fluid (GCF) volume and biomarkers.

Study design: Randomized Clinical Trials (RCTs) and Clinical Controlled Trials (CCTs).

Selection criteria

This review sought RCTs and a CCT comparing the effect of probiotics on experimental gingivitis in humans. Animal studies, retrospective cohort studies, *in vitro* studies, case series, case reports, and reviews were excluded. Moreover, studies conducted on children or teenagers, peri-implant diseases, active gingivitis and/or periodontitis, and no hygiene interruption were also excluded.

Screening process

Two independent reviewers (C.M. and P.A.) conducted the search and screening process. Analysis

Table 1. Systematic search strategy (PICO).

Parameter	Search strategy
Population	"Humans" [MeSH Terms] OR "Humans" [Text Word] OR "Adults" [MeSH Terms] OR "Adults" [Text Word] "Oral Health" [MeSh Terms] OR "Oral Health" [Text Word] OR "Gingivitis" [MeSh Terms] "Gingivitis" [Text Word] OR "Experimental" [All Fields]
Intervention	"Probiotic" [MeSH Terms] OR "Probiotic" [Text Word] OR "Therapy" [subheading] OR "Therapeutics"[MeSH Terms] OR "Therapy" [Text Word]
Comparisons	"Placebo" [MeSH Terms] OR "Placebo" [Text Word] OR "Lactobacillus" [MeSH Terms] OR "Lactobacillus" [Text Word] OR "Cultured milk product" [Mesh Terms] OR "Cultured milk product" [Text Word] OR "Ice cream" [Mesh Terms] OR "Ice cream" [Text Word] OR "Cheese" [Mesh Terms] OR "Cheese" [Text Word] OR "Yogurt" [Mesh Terms] OR Yogurt [Text Word] OR "Lozenge" [All Fields] "Tablets" [MeSH Terms] OR "Tablets" [Text Word] OR "Biofilm" [MeSH Terms] OR "Biofilm" [Text Word] OR "Dental Plaque" [MeSH Term] OR "Dental Plaque" [Text Word] OR OR "Colonization" [All Fields] OR "Microbiota" [MeSH Terms] OR "Microbiota" [Text Word] OR "Cytokines" [MeSH Terms] OR "Cytokines " [Text Word]
Outcomes	"Disease" [MeSH Terms] OR "Disease" [Text Word] OR "Activity [Text Word] OR "Inflammation" [MeSH Terms] OR "Inflammation" [Text Word] OR "Bleeding" [Text Word] OR "Immunomodulation" [MeSH Terms] OR "immunomodulation" [Text Word] OR "Biomarkers" [MeSH Terms] OR "Biomarkers" [Text Word]
Study design	Randomized Clinical trial and Controlled Clinical trial: Follow up until 1 year.
Search combination	#1 AND #2 AND #3
Database search	March to July 2018
Language	English
Electronic database	PubMed/MEDLINE, Cochrane Central Register of Controlled Trials, Web of Science and Trip, Grey literature

of titles and abstracts was performed, followed by full-text article selection, analyzed according to eligibility criteria (inclusion/exclusion). Possible disagreements were resolved in concession meetings.

Quality assessments

The risk of bias was independently assessed by two authors (E.B. and K.V.) using the Cochrane Collaboration's tool.[19] The analysis of each study was based on the following six criteria: sequence generation, allocation concealment, blinding, incomplete outcome data, selective outcome, and other sources of bias. Studies were rated at low, medium, or high risk of bias, when they met all, all except one, or all except two or more criteria, respectively.

Data extraction

Data were extracted (by D.L. and K.V), and a standardized form was used to record the following: authors, study design, clinical parameters (bleeding on probing – BOP, plaque index – PI, gingival index – GI, probing depth – PD, interproximal plaque index – IPI, papilla bleeding index – PBI), gingival crevicular fluid volume – GCF, biomolecular parameters IL-1β, IL-6, IL-8, IL-10,IL-18, TNF-α, MIP-1β, matrix metalloproteinase-8 (MMP-8), prostaglandin E2 (PGE2), nitrite/nitrate, matrix metalloproteinase-3 (MMP-3), polymorphonuclear elastase (PMN elastase), myeloperoxidase (MPO), microbiological findings, stage of the disease, probiotic administration, follow-up, and mean difference between baseline and final follow-up.

Statistical analysis

The positive effect of probiotics and the follow-up period of the included studies were calculated by estimating the intervention that was expressed in mean difference (MD) and $p < 0.05$. In this review, there was no possibility of performing a meta-analysis, due to the considerable heterogeneity between the studies.

Results

Literature search

The initial search resulted in 192 titles from PubMed/MEDLINE, 0 titles from Cochrane Central

Register of Controlled Trials, 6 from Web of Science, and 47 from TRIP; a total of 245 titles. On the first title analysis, 123 duplicates were excluded. After abstract screening, 107 were excluded. Fifteen full-text articles were selected. After careful reading, ten studies were excluded as they failed to conform to the eligibility criteria of this review. Finally, five studies published between 2009 and 2017, were included in this systematic review (Figure).[9, 20,21,22,23]

Additional analysis

The k concordance value for the two reviewers was 100% for the potential articles to be included (titles and abstracts) and for the articles selected; this indicated substantial concordance for the potential articles and 'perfect' agreement, k=1.[14]

Study characteristics

The characteristics of the included studies are presented in Table 2. Four studies were RCTs[9,20,21,22] and one study was a CCT[23] comparing the use of probiotics (test group) with placebo (control group). The number of participants ranged from 18[20] to 51,[9] with a mean of 36.2 participants. The mean follow-up period was 11.6 days (range 4-21 days). The included studies assessed whether daily oral administration of probiotic bacteria could influence the inflammatory response and the composition of supragingival plaque in an experimental gingivitis model. All studies evaluated PI and GCF;[9,20-23] three studies assessed PI, BOP, GI, and GCF;[20,21,22] one study evaluated PBI[9] and IPI; and one study evaluated PD.[22] The test groups from two studies received a milk drink containing *Lactobacillus casei* Shirota,[9,23] two

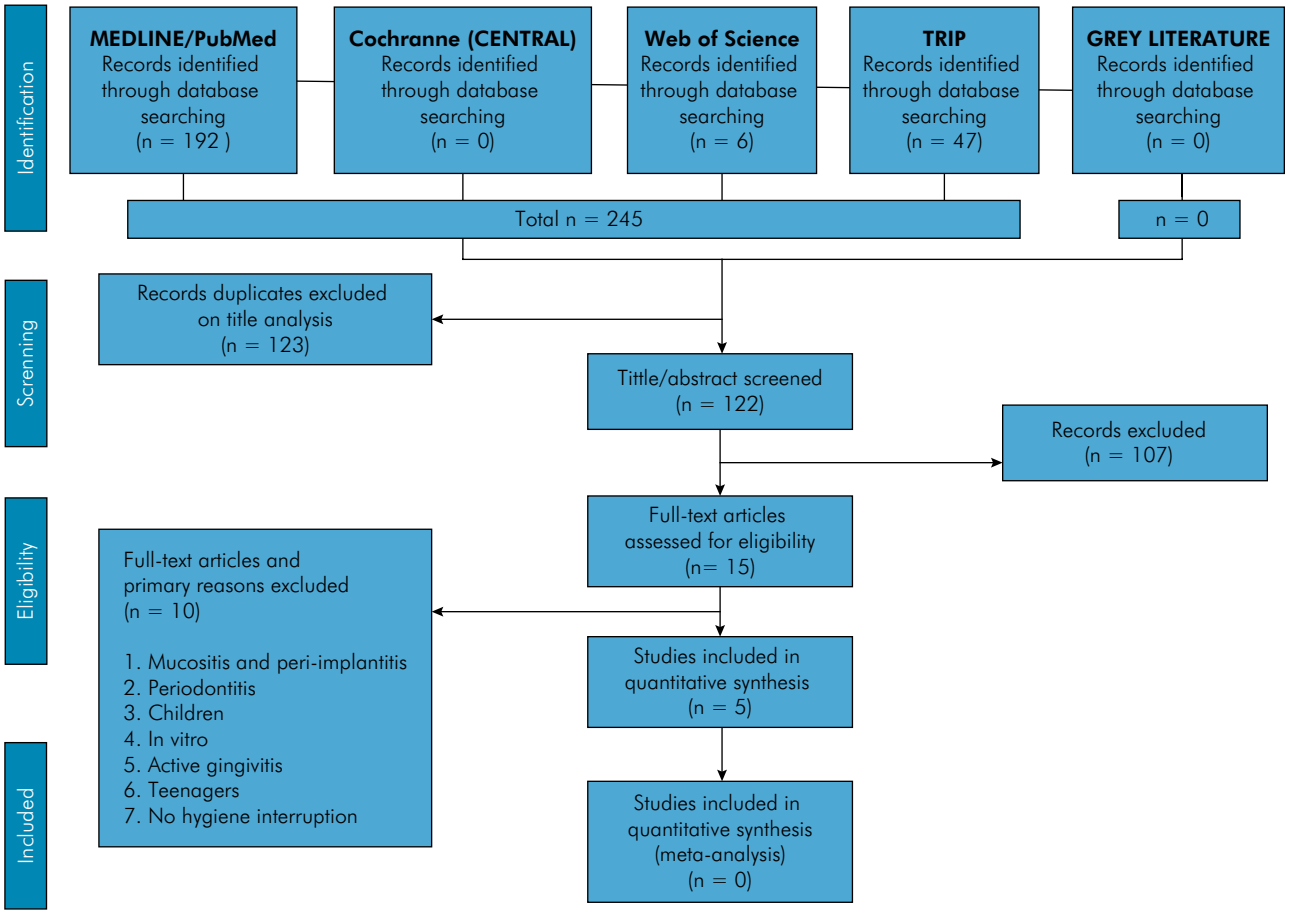

Figure. Flow diagram of the screening and selection process.

Table 2. Included studies.

First Author	Staab B.	Slawik S.	Hallström H.	Lee JK.	Kuru BE.
Year of publication	2009	2011	2013	2015	2017
Study Design	RCT	CCT	RCT	RCT	RCT
Characteristics of the participants	n = 50 Test: 25 (male/female 12/13) Control: 25 (male/female 12/13) Age range 24.4 years	n = 28 Test: 14 Control: 14 Age range 27.5 years	n = 18 Test: 9 (female) Control: 14 Control: 9 (female) Age range 38 years	n = 34 Test: 14 (male/female 9/5) Control: 16 (male/female 11/5) 4 participants were lost Age range Test/Control 22.1/21.6	n = 51 Test: 26 (male/female9/17) Control: 25 (male/female 10/15) Age range Test/Control 22.8/21.64
Probiotics	Lactobacillus casei Shirota	Lactobacillus casei Shirota	Lactobacillus reuteri	Lactobacillus brevis CD2	Bifidobacterium animalis subsp.
Administration methods	Milk drink (Yakult®) (65 ml once a day)	Milk drink (Yakult®) (65 ml once a day)	Lozenge (1) Twice a day	Lozenge (1) 3 times a day	Yogurt (110g/day)
Time of experiment	8 weeks + 4 days	4 weeks	3 weeks	2 weeks	4 weeks + 5 days
Experimental gingivitis	Mechanical hygiene interruption 4 days after a period of 8 weeks of probiotic intake	Mechanical hygiene interruption 14 days after 2 weeks of probiotic intake	Use of a stent to prevent brushing during 3 weeks of probiotic intake	Mechanical hygiene interruption 14 days during the probiotic intake	Mechanical hygiene interruption 5 days after a period of 4 weeks of probiotic intake
Data collection	After 8 weeks of probiotic consumption (baseline) and 96 hours later (final)	1 (baseline), 3, 5, 7, and 14 days (final)	0 (baseline) and 21 days (final)	0 (baseline), 3, 7, 10, and 14 days (final)	0 (baseline), 28 and 33 days (final)
Clinical parameters/ Biomarkers	PI, PBI, IPI, GCF, MOP, PMN – elastase(NE), MMP-3	PI, GI, BOP, GCF volume	PI, GI, BOP, GCF, IL-1β, IL-6, IL-8, IL-10, IL-18, TNF-α, MIP1-β	PI, GI, BOP, GCF, MMP-8, PGE2, NO	PI, GI, PD, BOP, GCF volume, IL-1β
Final Outcomes (mean difference ± SD, ρ-value)	PI: test: 2.52±0.61 control: 2.14±0.30 ρ < 0.0001 PBI: test: 1.17±0.57 control:1.12 ± 0.36 ρ < 0.001-0.003 IPI: test: 9.31±8.8 control: 96.6±8.0 ρ < 0.001 GCF biomarkers: MOP: test: 3.58±5.27 control: 8.50 ± 8.00 ρ = 0.014 PMN-elastase (NE): test: 0.0198±0.0117 control: 0.0430 ± 0.0387 ρ = 0.001 MMP-3: test: 27.03±8.59 control: 30.21 ± 11.00 ρ < 0.001	PI: test: 2.14±0.56 control: 2.2 ± 0.32 ρ < 0.001 GI: test: 1.29±0.42 control: 1.16 ±0 .42 ρ < 0.0001 BOP%: test: 8.25±12.72% control: 23.76 ± 11.9% ρ = 0.005 GCF (PU): test: 18.78 ± 16.7 control: 35.72 ± 16.1 ρ = 0.005	*PI: test: 18 control: 18 ρ < 0.05 *GI: test: 15 control: 14 ρ < 0.05 *BOP: test: 18 control: 17 ρ < 0.05 **GCF(μL): test: 0,25±0.30 control: 0.22 ± 0.24 ρ < 0.05 IL-1β: test: 76.6 ± 70.2 control: 60.5 ± 65.4 ρ < 0.05 IL-6: test: 5.15 ± 16.2 control: 1.58 ± 2.45 ρ NS IL-8: test: 36.8 ± 34.0 control: 33.4 ± 27.5 ρ < 0.05 IL-10: test: 0.43 ± 0.46 control: 0.38 ± 0.26 ρ NS IL-18: test: 98.6 ± 105.7 control: 116.2 ± 112.1 ρ <0.05 TNF-α: test: 1.45 ± 4.14 control: 0.66 ± 1.03 ρ NS MIP1-β: test: 7.8 ± 11.3 control: 5.5 ± 2.3 ρ < 0.05	PI: test: 1.44 ± 0.27 control: 1.41 ± 0.31 ρ < 0.001 GI: test: 0.25 ± 0.11 control: 0.2 5± 0.11 ρ = 0.001 ***BOP: test: 16.71 ± 7.76 control: 16.88 ± 9.75 ρ = 0.194 GCF biomarkers: MMP-8: test: 2688.6±2373.9 control: 2495.6 ± 2656.1 ρ NS PGE2: test: 3.41 ± 4.80 control: 2.42 ± 4.08 ρ NS NO: test: 9120.2 ± 4094.5 control: 10651.5 ± 6027.3 ρ = 0.05	PI: test: 0.28 ± 0.14 control: 0.37 ± 0.15 ρ < 0.001 GI: test: 0.80 ± 0.33 control: 1.52 ±0.44 ρ < 0.001 BOP: test: 11.87 ± 4.12 control: 22.81 ± 6.12 ρ < 0.001 PD: test: 1.44 ± 0.15 control: 1.58 ± 0.12 ρ NS GCF (μL): test: 0.19 ± 0.04 control: 0.14 ± 0.04 ρ = 0.101 IL-1β: test: 0.09 ± 0.07 control: 0.63 ± 0.44 ρ < 0.001
Conclusions	A beneficial effect of probiotic milk drink on gingival inflammation.	Daily consumption of a probiotic dairy drink reduces the effects of induced gingival inflammation associated with a higher plaque score due to the high carbohydrate content of probiotic milk.	There was no difference between groups.	L. brevis CD2 delays the development of gingivitis by regulating the inflammatory cascade.	Positive effect on gingival parameters in the probiotic group and reduction of clinical and immunological inflammation.

RCT: randomized clinical trial, CCT: controlled clinical trial, PI: plaque index, GI: gingival index, IPI: interdental plaque index, BOP: bleeding on probing, PD: probing depth, GCF volume: gingival crevicular fluid volume, MOP: myeloperoxidase, MMP-3: matrix metalloproteinase-3, PMN-elastase (NE): polymorphonuclear elastase, TNF-a: tumor necrosis factor – alpha, Interleukins (1β, 6, 8, 10, 18), MIP1-β: Macrophage inflammatory protein 1- beta, PGE2: Prostaglandin E2, NO: nitric oxide, NS: not significantly different.
* No showed differences in PI, GI, or BOP between the groups.

studies received lozenges containing *Lactobacillus reuteri* and *Lactobacillus brevis* CD2,[20,21] and one study received Yogurt containing *Bifidobacterium animalis* subsp. *lactis* DN-173010.[22]

The studies conducted by Hallstrom et al.[20] and Lee et al.[21] evaluated the following clinical parameters: PI, GI, and BOP. In both studies, the results showed that there was an increase in PI, IG, and BOP from the baseline to the final follow-up, with no inter-group difference. However, Lee's study revealed that BOP was higher in the placebo group than in the probiotic group.[21] In both studies, the subjects abstained from oral hygiene for two weeks.[20,21] In the study by Lee et al.,[21] the participants refrained from tooth brushing, while Hallstrom et al.[20] used an acrylic stent on the teeth involved in the study to prevent accidental cleaning.

Studies by Kuru et al.[22] and Slawik et al.[23] have shown similar results for PI and GI. The indices showed a comparable increase in test and control groups. However, the BOP showed a significant increase in the control group. Kuru et al.[22] was the only study that evaluated PD and found an increase in the control group. Staab et al.[9] evaluated the interproximal plaque index (IPI) and, unlike the other studies, concluded that there was greater bleeding in the test group.

All studies included in this review showed a smaller change in GCF volume in the presence of probiotic.[9,20,21,22,23] However, four studies[9,20,21,22] analyzed specific biomarkers of inflammation, such as MMP-8, prostaglandin E2, nitrite/nitrate conversion,[21] PMN-elastase, MPO and MMP-3,[9] IL-1ß,[22] IL-1ß, IL-6, IL-8, IL-10, IL-18, TNF-α, and MIP-1ß.[20] In the evaluation of the biomarkers IL-1B, IL-18, MMP-3, and MPO, three studies showed increased fluid volume in the control group when compared to the test group.[9,20,22] The concentrations of the biomarkers MIP-1ß, IL-8,[20] and prostaglandin E2[21] were lower in both groups between the baseline and the final follow-up. However, the biomarkers TNFα, IL-6, IL-10[20], MMP-8,[21] and PMN- elastase[9] showed no intergroup difference.

One study examined the changes in the microbiological profile of supragingival plaque and concluded that the number of bacteria increased in both groups, mainly *Fusobacterium nucleatum* and *Veillonella parvula*. Additionally, the concentration of *Streptococcus oralis* was higher only in the test group. The bacteria *Tannerella forsythia*, *Streptococcus mutans*, and *Lactobacillus fermentum* were hardly identified in the samples.[20]

Quality analysis

The quality analysis of RCTs and CCT included in the study are shown in Table 3. Five studies showed a low risk of bias,[9,20-23] two studies[20,22] met all the criteria described in the Cochrane Collaboration's tool,[14] and three studies scored negatively,[9,21,23] one in each question. No study used the CONSORT statement guidelines.[24]

It is important to emphasize that the ELISA technique used by Staab et al.[9] and Kuru et al.[22] to analyze biomarkers may have been compromised by the low sensitivity of the method and the limited amount of fluid. Hallström et al.[20] used the DNA-DNA Checkerboard hybridization method to analyze existing bacteria. Although this technique is quick

Table 3. Quality assessment of included studies.

First author and year of publication/ Quality assessment	Staab et al.[9] 2009	Slawik et al.[23] 2011	Hallström et al.[20] 2013	Lee et al.[21] 2015	Kuru[22] et al. 2017
Adequate sequence generation	Yes	Yes	Yes	Yes	Yes
Allocation concealment	Yes	No	Yes	Yes	Yes
Blinding	No	Yes	Yes	Yes	Yes
Incomplete outcome data addressed	Yes	Yes	Yes	No	Yes
Selective outcome reporting	Yes	Yes	Yes	Yes	Yes
Free of other sources of bias	Yes	Yes	Yes	Yes	Yes

and sensitive, it may demonstrate false-positive results leading to a risk of bias.

Discussion

This study systematically reviewed randomized clinical trials and a controlled clinical trial on the effect of probiotics on experimental gingivitis. The included studies showed different results.[9,20,21,22,23] It was necessary to interrupt mechanical oral hygiene procedures to evaluate the parameters indicative of the host inflammatory response, such as the bleeding frequency of the gingiva and the gingival crevicular fluid volume.[23] Lee et al.[21] advised the volunteers to discontinue tooth brushing. Hallström et al.[20] used an acrylic stent on the involved teeth to prevent accidental cleaning. This methodology may have influenced the clinical results obtained because of stent inadequacies or biofilm disruption by tooth brushing in adjacent areas.

All studies highlighted the immediate effects of probiotics during the non-brushing period.[9,20,21,22,23] However, for probiotics to be effective in treatment or prevention, a minimum concentration of $1x10^8$ CFU should be administered.[25] In addition, the administration of probiotic can affect its immunomodulatory effect, since a constant issue in the development of functional foods is the functionality of bioactive cultures.[26]

Two studies[20,21] used probiotic-containing lozenges, one contained *Lactobacillus reuteri* (ATCC55730 and ATCC PTA5289; $1×10^8$ CFU of each strain) twice daily,[20] and another contained *Lactobacillus brevis* CD2 three times a day.[21] Only one study used a milk drink (Yogurt) containing $≥10^8$ colony forming units (CFU)/g *Bifidobacterium animalis* subsp., once a day.[22] Two other studies used a dairy drink containing *Lactobacillus casei* Shirota.[9,23] The studies that used probiotics in the form of milk drinks showed better results with delayed development of gingivitis, demonstrated as a reduction of papillary bleeding and bleeding on probing, in addition to a moderate increase in GCF volume.[9,22,23] However, they showed a comparable increase in biofilm in both groups, an observation that can be explained by the amount of carbohydrate present in the probiotics.[9,22,23] The use of probiotic lozenges showed less expressive differences between groups.[20,21] The survival of probiotic microorganisms can be affected by several factors such as the composition of the food matrix, pH, carbon source, exposure to oxygen, and variation of the time-temperature binomial during processing and storage.[26,27] In the present review, none of the studies evaluated the technological aspects of the lozenges and the viability of the probiotic microorganisms during the storage period. Our review suggested that the probiotic drink showed better results on experimental gingivitis than the probiotic lozenges.[9,20,21,22,23] Functional foods, such as yogurt and milk, improved survival of microorganisms during the storage and the fermentation process enabling higher counts of probiotics in food.[12,26]

All studies presented differences in clinical response to probiotics due to the form of administration, dosage, time, and probiotic strains.[9,20-23] Hallström et al.[20] concluded that there was no difference in the clinical levels of IP, GI, and BOP between the groups. The study by Lee et al.[21] used lozenges and found less bleeding in the placebo group, while the other clinical parameters did not differ. However, in the studies by Staab et al.,[9] Kuru et al.,[22] and Slawik et al.,[23] PI, GI, and BOP were lower in the test group, proving that the probiotic produced positive clinical results. It is important to emphasize that gingival bleeding is a sensitive and reliable clinical indicator of gingival inflammation.[28]

The inflammatory response was also assessed by GCF volume and its biomarkers. The study conducted by Slawik et al.[23] was the only one to evaluate fluid volume.[23] The results were comparable to other studies,[9,20,21,22] in which there was a significant increase in fluid volume in the control group. The IL-1ß, IL-18, MMP-3, and MPO biomarkers showed higher levels in the placebo group than in the test group. Other biomarkers such as TNFα, IL-6, IL-10, MMP-8, and PMN elastase did not present differences between the groups.[9,20,21,22] The GCF biomarker concentrations indicated a positive probiotic effect on the immunomodulatory host response. The results of the studies suggested that probiotics delayed the development of experimental gingivitis.[9,21,22,23]

The only study that evaluated the microbiological profile of supragingival plaque showed that the

number of bacteria increased in both groups. Bacteria *Streptococcus oralis* and *Actinomyces naeslundii* were the most prevalent. *Fusobacterium nucleatum* and *Veillonella parvula* grew in both groups. *Streptococcus oralis* grew only in the probiotic group, while *Tannerella forsythia*, *Streptococcus mutans*, and *Lactobacillus fermentum* were hardly identified in both groups.[20] Although the study by Kuru et al.[22] did not perform a microbiological analysis, the antimicrobial properties of Bifidobacteria could have influenced the composition of the biofilm by inhibiting the periodontopathogens during the period of non-brushing. In contrast, a recent systematic review observed that probiotic bacteria attach to the oral tissues more strongly than pathogens, being able to compete for adhesion surfaces, thus producing a new biofilm. The authors concluded that probiotic use benefits the maintenance of oral health by decreasing the number of colony-forming units (CFU) of the oral pathogens.[29]

All studies included in this SR concluded that biomarker response patterns and modulation of inflammatory cytokines indicated a beneficial effect of probiotics in host response regulation, which is evidenced even with a gradual increase of plaque.[9,20,21,22,23]

The results of this review should be interpreted with caution. The RCTs and CCT included in this study had short follow-up periods. Additionally, the studies did not analyze the microbiome. The quantitative and qualitative method of analysis offers greater accuracy in the evaluation of beneficial and harmful periodontal bacteria. Studies based on microbiome analysis techniques may explain dysbiosis and the anti-inflammatory action of the probiotics.

Conclusions and clinical implications

The results of the analyzed studies using probiotics in experimental gingivitis showed a slight improvement in clinical parameters. The GCF volume significantly decreased in the presence of probiotics in experimental gingivitis compared to the placebo group. The results indicated that the positive effect of probiotics were due to the modulation of the host response, not the anti-plaque effect. However, the available evidence presented heterogeneity between the type of study, type of probiotic, dosage, administration method, and non-brushing period. Therefore, randomized clinical trials are needed to elucidate the mechanisms of probiotic action and develop better delivery systems.

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