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Probiotic supplements and debridement of peri-implant mucositis: A randomized controlled trial

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Abstract

Objective. The aim of this double-blind randomized placebo-controlled trial was to evaluate the effects of probiotic supplements in adjunct to conventional management of peri-implant mucositis. ***Materials and methods.*** Forty-nine adult patients with peri-implant mucositis were consecutively recruited after informed consent. After initial mechanical debridement and oral hygiene instructions, the patients received a topical oil application (active or placebo) followed by twice-daily intake of lozenges (active or placebo) for 3 months. The active products contained a mix of two strains of *Lactobacillus reuteri.* Patients were clinically monitored and sampled at baseline and after 1, 2, 4, 12 and 26 weeks. The clinical end-points were pocket-probing depth (PPD), plaque index (PI) and bleeding on probing (BOP). In addition, the subgingival microbiota was processed with checkerboard DNA-DNA hybridization and samples of gingival crevicular fluid (GCF) were analyzed for selected cytokines with the aid of multiplex immunoassays. ***Results.*** After 4 and 12 weeks, all clinical parameters were improved in both the test and the placebo group. PPD and BOP were significantly reduced compared with baseline ($p < 0.05$), but no significant differences were displayed between the groups. The clinical improvements persisted 3 months after the intervention. No major alterations of the subgingival microflora were disclosed and the levels of inflammatory mediators in GCF did not differ between the groups. ***Conclusions.*** Mechanical debridement and oral hygiene reinforcement resulted in clinical improvement of peri-implant mucositis and a reduction in cytokine levels. Probiotic supplements did not provide added benefit to placebo.

Key Words: *Cytokines, gingival crevicular fluid, lactobacilli, lozenges, subgingival bacteria*

Introduction

Peri-implant mucositis is a prevalent condition defined as a reversible inflammation of the peri-implant soft tissues not including alveolar bone loss [1,2]. The clinical features are swelling, redness and bleeding upon probing of the peri-implant soft tissues. It is generally thought that subgingival microbiota involved in peri-implant mucositis is similar to that of gingivitis and periodontitis [3]. This has been supported by experimental studies comparing development of inflammation at teeth and implants [4–6]. The non-surgical methods to treat peri-implant mucositis are based on mechanical debridement or mechanical debridement supplemented with antibacterial measures such as chlorhexidine, triclosan, ozone and/or hydrogen peroxide and systemically or locally administered antibiotics [7–13]. Although such strategies have been reported to be effective, the treatment does not result in a complete resolution of the inflammation in all cases [11]. Thus, there is room for improvement and novel treatment strategies. Probiotics are defined as 'live microorganisms which when administered in adequate amounts confer a health benefit on the host' [14]. Probiotic bacteria distributed in lozenges as adjunctive to conventional treatment of periodontitis and gingivitis has previously been shown to enhance healing and reduce

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the number of periopathogens in saliva [15–17]. Therefore, it was of interest to investigate if probiotics would have any additive effect to oral hygiene instructions and mechanical treatment (conventional treatment) of peri-implant mucositis. The aim of this study was to evaluate the effect of probiotic bacteria, applied as topical oil in the gingival sulcus and in lozenges for 3 months, on clinical variables adjacent to implants with a clinical diagnosis of peri-implant-mucositis. Secondary end-points were effects on inflammatory mediators in gingival crevicular fluid and composition of the subgingival microbiota. The research question was: Do probiotics, supplemented as lozenges and oil, have any additive effect to mechanical treatment on clinical parameters, microbiota and crevicular fluid around implants with peri-implant mucositis?

Materials and methods

Study outline

The investigation was performed from 2011–2013 and conducted as a double-blind randomized placebo-controlled trial with 3 months duration. All the participants signed a consent form and the Regional Ethical Review Board, Lund, Sweden, approved the study. The allocation to the test or placebo group was conducted using the Excel randomization tool in Microsoft Office.

Study group

Out of 53 eligible patients with peri-implant mucositis, 49 gave their informed consent and were consecutively enrolled in the study. The age range was 24–85 years. The inclusion criterion was one or more peri-implant sites with probing depth ≥ 4 mm combined with bleeding and/or pus on probing using a probing force of 0.2 N. Exclusion criteria were (i) bone loss exceeding 2 mm on recent radiographs as compared to radiographs exposed at the prosthetic delivery, (ii) patients with poorly controlled diabetes, (iii) pregnant or breast feeding women and (iv) patients taking systemic antibiotics or anti-inflammatory drugs within the last 3 months. Two patients in the test group were treated with antibiotics before the 6-month follow-up and another received prednisone, so these data were excluded in the final analysis. Thus, 22 subjects in the test group and 24 in the control group completed the entire protocol. The characteristics of the study groups are presented in Table I and a flowchart is shown in Figure 1.

Table I. Baseline characteristics of the enrolled subjects.

Variable	Placebo (n = 25)	Test (n = 24)
Mean age, years (SD)	63.3 (17.2)	53.7 (19.6)
Gender, female/male, n	14/11	17/7
Smokers, yes	8%	29%
Healthy, yes	64%	33%
Number of prescribed drugs (SD)	1.1 (1.8)	1.8 (1.8)
Number of implants per patient (SD)	4.0 (2.3)	2.5 (1.7)
Study implant, max/mand (n)	17/8	14/10

Clinical procedures

Before the start of intervention, all patients were given oral hygiene instruction and a comprehensive periodontal treatment. Mechanical debridement using titanium curettes and polishing using a rubber cup and polishing paste were performed. One implant with peri-implant mucositis (probing depth ≥ 4 mm combined with bleeding and/or pus on probing) in each patient was selected for the study. In the test group, the treatment session was concluded by a professional topical application of a droplet of an experimental oil containing *Lactobacillus reuteri* strains DSM 17938 and ATCC PTA 5289 (2×10^7 colony forming units of each strain, Prodentis, BioGaia AB, Lund, Sweden) around the selected implant. The oil was applied sub- and supragingivally with a ball-ended probe. The patients were then provided with commercial lozenges containing the same bacteria (1×10^8 CFU of each strain, ProDentis, BioGaia AB) and were instructed to let one sucking tablet slowly melt in the mouth twice daily (morning and evening). The control group got the same mechanical debridement and oral hygiene instructions and was treated with placebo oil and given placebo lozenges. The placebo and the active products were supplied in color-coded boxes and the lozenges had identical appearance, texture and taste. The intervention period was 3 months and follow-ups were performed at 1, 2, 4, 12 and 26 weeks after baseline. Oral hygiene re-instructions were given at the follow-ups if required. The patient, the therapist and the examiner were blinded for the group allocation. An independent monitor kept track of the randomization, delivered the oil and the lozenges to the therapist and was the only person with access to the data of the randomization throughout the entire project, including the statistical analyses. The compliance with the study protocol was regularly checked at the follow-ups and patients were instructed to return all non-consumed tablets.

Clinical recordings were performed at baseline and after 4, 12 and 26 weeks. The registrations were performed at four sites of each selected implant (buccal, mesial, lingual, distal) and included plaque index (PI), bleeding on probing (BOP), pocket probing depth (PPD) and presence of pus as described earlier [12]. Samples of crevicular fluid and bacteria were

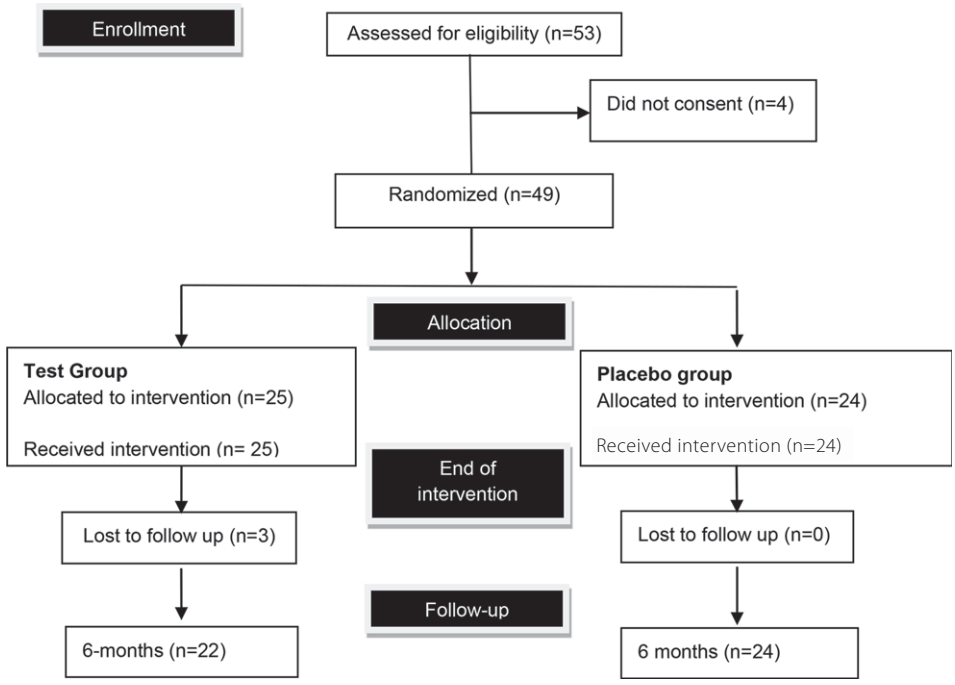

Figure 1. Flow-chart for participants and dropouts.

collected at baseline and after 1, 2, 4, 12 and 26 weeks. In addition, the most severe site of each implant (deepest PPD) was isolated with cotton rolls and supra-gingival plaque was removed with sterile cotton pellets. Paper points (Dentsply Maillefer size 55, Ballaigues, Switzerland) were inserted into the selected pocket until resistance was met and left *in situ* during 20 s. The volume was recorded using a Periotrone 8000 (ProFlow, Amenityville, NY) and the samples were then placed in labelled Eppendorf tubes (1.5 ml natural flat cap micro-centrifuge tubes; Starlab, Ahrensburg, Germany) containing 0.15 ml TE (10 mM Tris-HCL, 1 mM EDTA, pH 7.6). Within 30 min of sampling, vials were stored at –79°C until further analysis. All sampling and clinical registrations were made by one trained and calibrated examiner (SL), but the intra-examiner reliability was not formally evaluated prior to this study.

Laboratory assays

The concentration of IL-1β, IL-1RA, IL-4, IL-6, IL-8, IL-17A, CCL5, TNF-α, IFN-γ and GM-CSF was determined in GCF samples using a commercial Bio-Plex Cytokine Assay (Bio-Rad Laboratories, Hercules, CA) according to the manufacturer's instructions for the xMAP technology with multiple beads and expressed as pg/ml. Plates were measured using the Bio-Plex MagPix System and analyzed with the Bio-Plex Manager (version 6.0; Luminex, Austin, TX). In brief, the collected samples were thawed and suspended in 200 µl PBS containing 0.5% BSA. The vials were then centrifuged at 10,000 g for 10 min at 4°C. Antibody-coupled magnetic beads were added to each 96 well plate and the plates were washed with Bio-Plex wash buffer (2100 µl). The content of the peri-implant GCF samples were then pipetted and added in duplicate. After 30 min of incubation, the samples were washed with buffer to remove unbound protein. A 25 µl aliquot of one concentration of Bio-Plex biotinylated detection antibody specific for a different epitope on the cytokine was added to each well, incubated (30 min) and subsequently washed with Bio-Plex wash buffer (3100 µl). The reaction mixture was detected by streptavidin-PE (10 min), followed by a Bio-Plex wash buffer (3100 µl). Beads were re-suspended in each well with 125 µl of Bio-Plex assay buffer and shaken on a plate shaker (1100 rpm, 30 s). Cytokine concentrations in the samples were calculated by Bio-Plex software using a standard curve derived from a recombinant cytokine standard, included in the 96-well plate.

The plaque samples were analyzed using the checkerboard DNA-DNA hybridization method [18] with respect to the 12 bacterial strains listed in Table IV. The obtained chemiluminescent signals were transformed to a score between 0–5 according to Papapanou et al. [19]. Score 1 ($\geq 10^4$ CFU) was selected as the cut-off level to contrast between colonized/non-colonized sites.

Statistical methods

All data were analyzed using the IBM-SPSS software (version 22.0, Chicago, IL). Descriptive statistics including mean values, standard deviations or distributions were calculated. Differences between baseline

and follow-up were compared within the groups with the aid of the Wilcoxon paired signed rank test. Differences between the groups with respect to continuous or categorized values were calculated with non-parametric tests and chi-square tests, respectively. A p-value < 0.05 was considered statistically significant.

Power analysis

The primary outcome measure was PPD and a 0.8 mm difference (SD = 1.0) between the study groups was considered to be clinically meaningful. A power calculation with $\alpha = 0.05$ and $\beta = 0.2$ indicated that 23 patients in each group would be required in order to avoid Type-I and Type-II errors, respectively.

Results

The study groups were slightly, but non-significantly, imbalanced with respect to general health and tobacco use at baseline; the proportion of smokers was higher in the test group, while the proportion of healthy patients was higher in the placebo group. The groups were, however, balanced regarding age and sex. Most of the implants studied were located in the maxilla. The results of the probing pocket depth are presented in Table II, while the plaque index PI and bleeding on probing are summarized in Table III. A general improvement was seen in both the test and placebo group at the follow-ups in comparison with baseline, but no significant differences were displayed between the groups. The change in PPD at the implant's deepest site ranged from 0.7–1.2 mm in both groups, which was significantly different compared to baseline ($p < 0.05$). Similarly, the plaque index and bleeding on probing was reduced, with over 50% in both groups during the intervention. The gingival condition was significantly improved compared to baseline ($p < 0.05$). Notable, the clinical improvements in terms of BOP and PPD persisted 3 months after the intervention was terminated.

Table II. Probing pocket depth (PPD, mean mm, SD) at baseline and at the follow-ups in the test (T, $n = 25$) and the placebo (P, $n = 24$) group.

Variable	group	BL	4 weeks	12 weeks	26 weeks[a]
Mean PPD at implant	T	4.3 (1.1)	3.8 (1.1)	3.7 (1.2)	3.7 (1.3)
	P	4.0 (1.4)	3.5 (1.4)	3.4 (1.4)	3.5 (1.5)
PPD, most severe site	T	5.1 (0.9)	3.9 (1.3)[b]	4.2 (1.3)[b]	4.0 (1.3)[b]
	P	5.2 (1.0)	4.5 (1.2)[b]	4.0 (1.3)[b]	4.2 (1.4)[b]

[a] data based on 22 subjects in the test group.
[b] statistically significant difference compared with baseline ($p < 0.05$), paired t-test.

Table III. Secondary clinical end-points at baseline and follow-up in the test (T, $n = 25$) and the placebo (P, $n = 24$) group.

Variable	Group	BL	4 weeks	12 weeks	26 weeks[a]
BOP at implant (yes, %)	T	54	20[b]	11[b]	14[b]
	P	58	27	18[b]	17[b]
PI at implant (yes, %)	T	26	10	9	12
	P	32	13	10	15
Pus at implant (yes, %)	T	13	2	2	2
	P	5	3	0	2

[a] data based on 22 subjects in the test group.
[b] statistically significant difference compared with baseline ($p < 0.05$).

Microbial findings

The microbial findings are presented in Table IV. The most prevalent strains were *F. nucleatum*, *P. micra*, *P. intermedia* and *P. nigrescens*, all commonly linked to periodontal disease. The same four species also displayed the highest counts. Likewise, *F. alocis* was highly prevalent, but only in low counts. *P. gingivalis*, *A. actinomycetemcomitans* and *T. denticola* were sparsely detected in all samples. No major alterations over time or differences between the groups were recorded.

Inflammatory mediators in GCF

The concentrations of the selected cyto- and chemokines in gingival crevicular fluid (CGF) are shown in Table V. The volume of GCF decreased in both groups during the intervention and at the follow-up ($p < 0.05$) compared with baseline. There was a tendency of reduced levels of the pro-inflammatory cytokines during the intervention period in both groups compared with baseline, but there were no statistically significant differences between the groups. After 4 weeks, the levels of IL-1RA, IL-8, CCL5, TNF-α and GM-CSF were significantly lower than baseline ($p < 0.05$).

Side effects and compliance

No complaints or harmful events following the intervention were registered during the trial. All patients were seen at least five times during follow-up and all claimed that they followed the protocol. According to interviews, the intake of the probiotic lozenges was only occasionally forgotten and in no case for several days in a row.

Discussion

Peri-implant mucositis is a reversible inflammatory condition confined to the peri-implant soft-tissues

Table IV. The prevalence (%) of selected bacterial strains (> 10^4 CFU) at baseline and follow-up in the test (T, $n = 25$) and the placebo (P, $n = 24$) group.

Strain, origin[a]	T/P BL	1 week	2 weeks	4 weeks	12 weeks	26 weeks[b]
Porphyromonas gingivalis	T: 8%	4%	4%	4%	8%	4%
(FDC381)	P: 4%	8%	4%	4%	8%	4%
Prevotella intermedia	T: 38%	38%	33%	21%	29%	30%
(ATCC26511)	P: 40%	28%	32%	40%	40%	48%
Prevotella nigrescens	T: 38%	33%	42%	46%	38%	39%
(ATCC 33563)	P: 48%	56%	44%	56%	64%	52%
Tannerella forsythia	T: 25%	21%	17%	21%	25%	23%
(ATCC43037)	P: 32%	20%	19%	12%	24%	32%
A. actinomycetemcomitans	T: 16%	8%	8%	16%	13%	9%
(FDC Y4)	P: 16%	4%	4%	8%	20%	12%
Fusobacterium nucleatum	T: 63%	46%	50%	50%	50%	43%
(ATCC10953)	P: 60%	60%	60%	64%	72%	64%
Treponema. Denticola	T: 17%	8%	4%	17%	13%	13%
(OMGS3271)	P: 32%	8%	20%	24%	20%	16%
Parvimonas micra	T: 58%	67%	67%	71%	54%	56%
(OMGS2852)	P: 56%	60%	48%	60%	64%	44%
Campylobacter rectus	T: 21%	17%	13%	21%	17%	13%
(ATCC33238)	P: 36%	28%	12%	16%	32%	20%
Porphymonas endodontis	T: 8%	4%	4%	4%	13%	13%
(OMGS1205)	P: 28%	12%	12%	12%	20%	24%
Filifactor alocis	T: 35%	38%	29%	25%	29%	35%
(ATCC35896)	P: 56%	48%	48%	64%	60%	52%
Prevotella tannerae	T: 29%	29%	17%	13%	21%	22%
(ATCC51259)	P: 44%	28%	32%	32%	32%	32%

[a] FDC, Forsyth Dental Collection, Boston, MA; ATCC, American Type Culture Collection; OMGS, Oral Microbiology, Gothenburg, Sweden.
[b] data based on 22 subjects in the test group.

and it is thought that effective treatment of peri-implant mucositis will prevent the development of peri-implantitis [20]. A main and important finding of this study was that mechanical debridement and oral hygiene instructions, with and without probiotic supplements, was a partly effective long-term treatment for this condition, thereby supporting the outcome of a previous report [11]. As all clinical variables improved over a 6-month period in both the test and the placebo groups, the null hypothesis could not be rejected. The reduction of BOP was obvious and statistically significant compared to baseline in both groups, while the general PPD improvement of ~ 0.5 mm did not reach statistical significance. The probing depth at the deepest pocket of each implant was, however, significantly improved. Thus, the probiotic therapy did not seem to increase efficacy. It is, however, important to underline that placebo-tablets also must be regarded as an intervention and should not be mixed-up with 'no treatment'. Teughels et al. [21] have previously reported significant clinical improvements following both probiotics and placebo as adjunct to scaling and root planning in the management of periodontitis. Others have claimed gingival and periodontal improvements exclusively after oral administration of various strains of probiotic bacteria [15,16,22,23]. Interestingly, neither the origin of the probiotic strain, nor the mode of administration seemed to influence the beneficial outcome in the above-mentioned studies. The present commercially available lozenges were well accepted by the patients and the compliance with the protocol was considered as good-to-excellent. No objective or subjectively perceived adverse-effects were reported. Most patients had a history of advanced periodontitis and seemed motivated not to further jeopardize the future of their installed implants.

The idea behind the topical applications of the probiotic oil was to locally alter the bacterial profile of the biofilm adjacent to the implants. *L. reuteri* has previously been shown to reduce selected periodontopathogens *in vivo* [21,24], but we found no major

Table V. Levels of selected inflammatory mediators (pg/ml) in GCF at baseline and follow-ups in the test (T, $n = 25$) and the placebo.

Variable	T/P: BL	1 week	2 weeks	4 weeks	12 weeks	26 weeks[a]
GCF (μL)[b]	T: 0.27 (0.22)	*0.07 (0.15)*[C]	*0.00 (0.07)*[C]	*0.03 (0.08)*[C]	*0.05 (0.11)*[C]	*0.07 (0.13)*[C]
	P: 0.31 (0.21)	*0.05 (0.10)*[C]	*0.07 (0.16)*[C]	*0.03 (0.08)*[C]	*0.05 (0.13)*[C]	*0.03 (0.10)*[C]
IL-1β	T: 14.2 (26.7)	19.2 (31.7)	8.0 (10.6)	7.7 (9.3)	11.8 (11.4)	13.6 (16.9)
	P: 17.1 (27.2)	21.4 (46.7)	13.2 (15.3)	7.2 (10.8)	15.4 (23,7)	8.2 (13.6)
IL-1RA[d]	T: 4.4 (4.5)	3.5 (5.0)	3.2 (4.5)	*2.5 (3.8)*[C]	5.5 (7.9)	3.5 (5.0)
	P: 7.1 (8.4)	11.8 (17.6)	6.2 (10–92)	*2.2 (3.1)*[C]	7.6 (11.3)	2.3 (2.9)[C]
IL-4	T: 2.7 (7.7)	0.6 (1.1)	0.4 (1.1)	1.6 (0.4)	2.1 (5.9)	0.5 (1.3)
	P: 1.2 (1.9)	2.1 (5.7)	0.4 (0.9)	0.4 (1.1)	0.8 (1.6)	0.8 (3.0)
IL-6	T: 7.2 (19.3)	14.2 (51.8)	1.3 (3.6)	1.2 (3.9)	3.4 (8.4)	2.5 (9.1)
	P: 2.8 (3.1)	2.6 (4.9)	*1.3 (1.9)*[C]	1.4 (4.9)	1.7 (2.6)	3.3 (10.1)
IL-8	T: 474 (670)	448 (782)	*201 (200)*[C]	*165 (160)*[C]	354 (310)	251 (246)
	P: 278 (297)	251 (243)	207 (195)	153 (295)	222 (240)	176 (249)
IL-17A	T: 11.3 (32.4)	1.1 (2.5)	1.0 (1.9)	0.9 (2.4)	5.8 (17.3)	1.3 (3.7)
	P: 3.9 (5.4)	6.2 (19.1)	3.0 (5.4)	*1.6 (3.7)*[C]	2.5 (5.4)	1.9 (5.6)
CCL5/	T: 8.6 (9.7)	*2.7 (4.9)*[C]	*22.3 (3.9)*[C]	*1.6 (3.6)*[C]	*3.5 (5.1)*[C]	*2.8 (5.5)*[C]
RANTES	P: 8.6 (8.0)	*4.5 (6.5)*[C]	*3.5 (4.8)*[C]	*1.8 (2.5)*[C]	*3.8 (6.2)*[C]	*3.2 (5.5)*[C]
TNF-α	T: 4.7 (10.0)	3.3 (5.4)	1.2 (1.6)	*0.8 (1.4)*[C]	2.3 (3.4)	1.2 (2.1)
	P: 2.7 (2.4)	2.8 (4.9)	2.5 (4.9)	*1.1 (1.8)*[C]	2.1 (3.1)	1.8 (2.8)
IFN-γ	T: 278 (792)	30 (80)	32 (84)	14 (46)	152 (435)	33 (64)
	P: 97 (124)	149 (462)	*42 (72)*[C]	*21 (45)*[C]	*43 (82)*[C]	36 (140)
GM-CSF	T: 1.6 (5.4)	0.1 (0.4)	*0.1 (0.3)*[C]	*0.1 (0.3)*[C]	0.7 (2.0)	0.1 (0.4)
	P: 0.2 (0.4)	0.9 (3.0)	0.6 (1.6)	0.3 (0.9)	0.1 (0.3)	0.4 (0.2)

[a] data based on 22 subjects in the test group.
[b] volume GCF.
[c] statistically significant difference compared with baseline (italic text), $p < 0.05$; paired t-test.
[d] expressed as pg/ml × 1000.

alterations in the subgingival microflora. A similar observation was recently made by Toiviainen et al. [25]. They showed that the periodontal status was improved without affecting the salivary oral microbiota, and this was understood in the context of earlier findings that probiotic lactobacilli may have a poor capacity to populate oral biofilms [26]. Interestingly, the present study group harbored low-to-moderate levels of the main pathogens associated with periodontitis, such as *P. gingivalis*, *T. denticola*, *T forsythia* and *A. actinomycetemcomitans*. Nevertheless, our results suggested that a single topical application of probiotic bacteria, followed by a daily oral administration of probiotic lozenges, did not affect the profile of the subgingival flora.

It has been suggested that regular intake of probiotic bacteria may exert an immune-modulation of the host [14]. Previous studies have indicated that a daily intake of tablets containing *L reuteri* reduced pro-inflammatory cytokines in the gingival crevicular fluid of adults with gingivitis [27] and chronic periodontitis [28]. We have previously shown increased volumes and levels of IL-1β and IL-18 in GCF during experimental gingivitis [29]. In this study the levels of IL-1β, IL-8, CCL5 and TNF-α were reduced by 40–50% compared with baseline after 4 weeks in both groups. Thus, it is likely that the reduction of the pro-inflammatory mediator response contributes to hamper the local peri-implant inflammatory process. Little is, however, known on the long-terms events and further studies in extended settings, up to 12 months, are warranted to further elucidate this process.

In conclusion, topical treatment and daily intake of probiotic lozenges as an adjunct to mechanical debridement and oral hygiene instructions did not improve clinical, microbial or inflammatory variables of peri-implant mucositis as compared to the use of placebo.

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