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The effect of orally administered probiotic *Lactobacillus reuteri*-containing tablets in peri-implant mucositis: a double-blind randomized controlled trial

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Background and Objectives: Probiotics create a biofilm and protect the oral tissues against the action of periodontal pathogenic bacteria. The aim of this study was to evaluate the effects of the oral probiotic *Lactobacillus reuteri* Prodentis upon the peri-implant health of edentulous patients with dental implants and peri-implant mucositis, establishing comparisons vs implants without peri-implant disease.

Material and Methods: A double-blind, placebo-controlled, prospective cross-over study was made. The patients were all edentulous and were divided into two groups, (A) no peri-implant disease, and (B) peri-implant mucositis affecting one or more implants. Patients with peri-implantitis were excluded. The dosage was one tablet every 24 h over 30 d. All patients in both groups initially received the oral probiotic *Lactobacillus reuteri* Prodentis, followed by placebo. Patients started with probiotic treatment during 30 d, followed by a 6 mo wash-out period and the administration of placebo for the same period. The following parameters were studied: crevicular fluid volume, modified plaque index, probing depth, modified gingival index, and concentrations of interleukin 1β, interleukin 6 and interleukin 8.

Results: A total of 77 implants were evaluated in 34 patients. Group A involved 22 patients with 54 implants without peri-implant alterations, and group B, 12 patients with mucositis affecting one or more implants (23 implants). After treatment with the probiotic, both the patients with mucositis and the patients without peri-implant disease showed improvements in the clinical parameters, with reductions in cytokine levels. In contrast, no such changes were observed with placebo.

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Conclusions: After treatment with the probiotic *Lactobacillus reuteri* in patients with implants presenting mucositis, the clinical parameters improved, and the cytokine levels decreased – in contraposition to the observations in the placebo group. Probiotic administration may be regarded as a good alternative for both the treatment of peri-implant mucositis and its prevention, as it also improved clinical parameters in the healthy individuals. Further studies involving larger patient series are needed regarding the effects of probiotics upon peri-implant health.

Introduction

Probiotics have been defined by the Food Agricultural Organization/World Health Organization (FAO/WHO) as live microorganisms that can offer health benefits (improving the microbiological balance of the intestine) when administered in adequate amounts (with meals or as dietetic supplements) (1). Within the oral cavity, probiotics create a biofilm and protect the oral tissues against cariogenic and periodontal pathogens, by occupying the space that the latter would tend to occupy (2,3).

The application of probiotics in the oral cavity can reduce the risk of high levels of *Streptococcus mutans* (4), and can offer benefits after application in periodontal treatments, reducing gingivitis and periodontitis (5); lowering the gingival index and the amount of bacterial plaque in patients with moderate or severe gingivitis treated with *Lactobacillus reuteri* (6); inhibiting the growth of *Porphyromonas gingivalis* and *Prevotella intermedia* (7); or reducing the concentrations of cytokines that mediate in inflammatory processes (8). Comparative studies of chlorhexidine rinses vs probiotic-containing rinses have found that although both groups showed reductions in certain clinical parameters, they were greater when probiotic-containing rinses were used (9). Probiotics can also be used to treat halitosis (10) and infections produced by *Candida albicans* (11), though a literature review on the use of probiotics in the oral cavity has yielded no studies on their effects in relation to peri-implant health (12).

Regarding the prevalence of peri-implant diseases, the literature offers controversial data. In any case, peri-implant disease is highly prevalent: 80% of all dental implant patients and 50% of all implants present peri-implant mucositis, and 28–56% of all dental implant patients and 12–43% of all implants present peri-implantitis, according to the study published by Zitzmann and Berglundh (13). The etiology of peri-implant disease infection has been described in detail in the literature for both mucositis (14,15) and peri-implantitis (16,17). Evaluation of the literature has shown the microbiota associated to peri-implantitis to be more complex than that found under healthy peri-implant conditions – the main flora consisting of anaerobic gram-negative bacteria (17). Crevicular fluid volume is one of the parameters used to diagnose peri-implant disease; specifically, an increase in crevicular fluid volume (18) can be related to the development of mucositis. Patients with mucositis have also been found to present an increase in crevicular fluid of the cytokine concentration (19,20). Cytokines include proinflammatory molecules such as interleukin (IL)-1β, IL-6 and IL-8, which control progression and/or suppression of the inflammatory response (21). Increased levels of IL-1β in crevicular fluid and in gingival tissue are associated with mucositis and peri-implantitis (22,23). The data suggest that IL-1β is significantly associated to the incidence of early marginal bone loss around endosseous implants (23). In turn, increased levels of IL-6 and IL-8 in crevicular fluid are related to the severity of mucositis and peri-implantitis (24–26). In a study of 34 individuals involving 77 dental implants, with 23 cases of mucositis and 54 healthy peri-implant sites, the mucositis group showed a significantly greater expression of IL-6 than the healthy group ($p < 0.05$) (26). In a review, Candel-Martí *et al.* (27) concluded that an increase in interleukin concentration is found in patients with peri-implant disease, though there is controversy regarding the effects of interleukins in crevicular fluid and peri-implantitis upon implant failure or the development of peri-implant disease.

To our knowledge, this study to analyze the effect of probiotics upon dental implants. It investigates the effect of the oral probiotic *Lactobacillus reuteri* upon the peri-implant health of edentulous patients with peri-implant mucositis, evaluating a series of clinical (modified plaque index, probing depth, modified gingival index) and immunological parameters (peri-implant crevicular fluid volume, peri-implant concentrations of IL-1β, IL-6 and IL-8), and establishing comparisons vs healthy patients with dental implants without peri-implant disease.

Material and methods

Study population

A double-blind, placebo-controlled, prospective cross-over study was made. Patients in the peri-implant maintenance phase during the period between January 2008 and June 2010 in the Oral Surgery and Implantology Unit of a University Hospital were included. The supervisor of the study (MPD) was the only person knowing which treatment was given to the patients, and coding was not unblinded until the end of the study. The clinical examiner (AFF) and the

patients were blinded. All patients presented at least one completely edentulous dental arch, with dental implant rehabilitation in one or both arches (TSA® implants, Avantblast® surface; Phibo Dental Solutions; Sentmenat, Barcelona, Spain).

The exclusion criteria were: (i) patients receiving any kind of local or systemic decontamination treatment of the oral cavity in the last 3 mo, or periodontal treatment in the last 6 mo; (ii) uncontrolled periodontal disease; (iii) patients with systemic disorders capable of influencing the treatment results; (iv) smokers; (v) incomplete protocols due to a lack of patient cooperation; (vi) failure to provide informed consent to participation the study; and (vii) patients presenting at least one implant with peri-implantitis (as established from the periapical X-ray study), defined by Schwarz *et al.* (28) as an implant with a probing depth of ≥ 4 mm and signs of acute peri-implantitis (loss of supporting bone as estimated on X-rays, bleeding on probing or suppuration), and no implant mobility.

The inclusion criteria were: (i) at least one completely edentulous dental arch subjected to dental implant rehabilitation; (ii) prosthetic restoration in function for at least 24 mo; (iii) healthy individuals without known disease; and (iv) non-smokers. The patients were divided into two groups according to whether they presented peri-implant mucositis or not. Based on the Consensus Report of the VII European Workshop in Periodontology (29), the implants with peri-implant gingival redness, swelling, bleeding on probing and without radiographic signs of bone loss were considered to present peri-implant mucositis. The inclusion criteria in the group of patients with healthy dental implants were probing depth < 4 mm (20,30), the absence of clinical signs of inflammation of the peri-implant mucosa, and no evidence of radiographic bone loss beyond bone remodeling. If one of the implants was healthy and the others showed signs of peri-implant mucositis, the patient was classified as having mucositis (31).

The study was conducted in accordance with the Declaration of Helsinki. The protocol was approved by the institutional review board of the Hospital, and the patients gave written informed consent to participation in the study. Registration number ClinicalTrials.gov: NCT01974596.

Study variables

The control visits were made by an appropriately trained and calibrated clinician (AFF) (Fig. 1) blinded to the timing of treatment. Data referred to age, gender and brushing frequency were collected (Table 1). The modified gingival index (mGI) (0: No bleeding when a periodontal probe is passed along the mucosal margin adjacent to the implant; 1: Isolated bleeding spots visible; 2: Blood forms a confluent red line on mucosal margin; and 3: Heavy or profuse bleeding) and the modified plaque index (mPI) (0: No detection of plaque; 1: Plaque only recognized by running a probe across the smooth marginal surface of the implant; 2: Plaque can be seen by the naked eye; and 3: Abundance of soft matter) were determined for each implant according to Mombelli *et al.* (32). The probing depth was determined using a soft plastic periodontal probe for implants with torque control at 0.25 N (Click-Probe®; Kerr, Bioggio, Switzerland). The peri-implant pockets of all the implants were recorded. Three reference points were taken vestibular and three lingual for each implant, with calculation of the mean probing depth in millimeters for each implant.

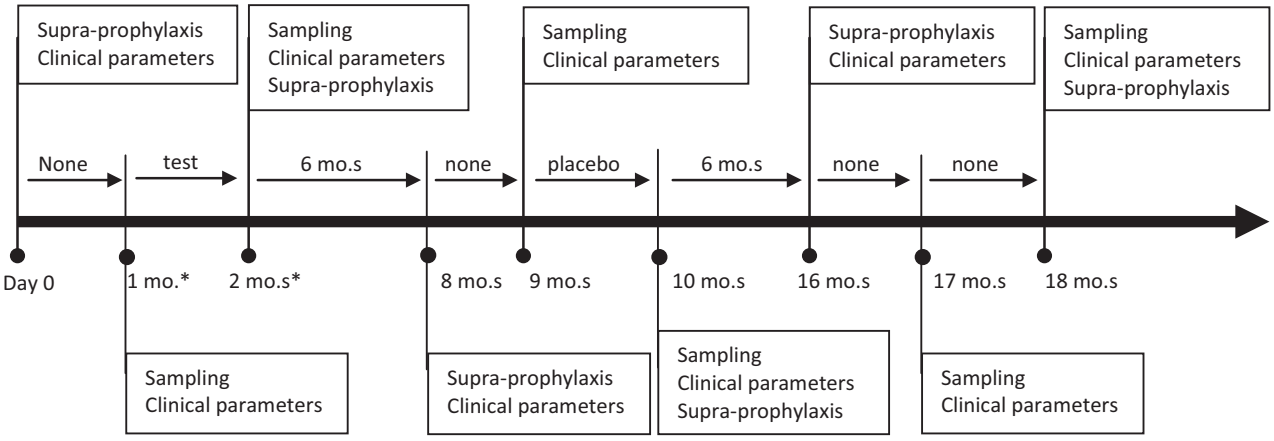
The implant with the deepest peri-implant pocket was selected for obtaining the samples for the determination of IL-1β, IL-6 and IL-8, selecting an implant from each rehabilitated quadrant. The clinical examiner selected the implant at the time of first supragingival prophylaxis. In the case of two or more implants with the same probing depth, the most anteriorly positioned implant was selected. Two samples were taken if the patient had restoration only of the mandible or the maxilla, while four samples were taken if the patient had restoration of both arches (registering two or four implants according to whether the upper maxilla, the mandible, or both were rehabilitated).

Radiological control

Parallelized intraoral X-rays were used to measure marginal bone loss of the dental implants. The X-ray study was carried out with the XMind® intraoral system (Groupe Satelec-Pierre Rolland, Bordeaux, France) and the RVG® intraoral digital receptor (Kodak Dental System, Atlanta, GA, USA). The XCP® X-ray positioning device (Dentsply, Des Plaines, IL, USA) was used to reproduce the angle of the X-rays in posterior reviews. To position the XCP® correctly, the guide bar was placed parallel to the direction of the X-ray beam, perpendicular to the digital receptor. According to the VII European Workshop in Periodontology, to establish baseline, a radiograph should be obtained to determine alveolar bone levels after physiological remodeling, and peri-implant probing assessments should be made. It is assumed that bone loss occurring after initial remodeling is mainly due to bacterial infection (29).

Peri-implant crevicular fluid sampling

Peri-implant crevicular fluid volume was recorded for all the dental implants before performing the respective clinical measurements, to avoid interference with the values (33). Sampling was carried out by a single trained and calibrated operator (AFF), using sterile paper strips (Periopaper Strip®; Proflow Incorporated, New York, NY, USA). The technique was performed as follows: (i) drying of the mouth with aspiration; (ii) isolation of the zone using cotton rolls; (iii) elimination of supragingival plaque from the sampling zone; (iv) gentle drying of the implant zone where the paper strip is placed; (v) collection of the sample of crevicular fluid by placing Periopaper® in the sulcus between the implant and gums during 30 s; (vi) reading of the sample with the Periotron® 8000



***mo.:** month; **mo.s:** months; **none:** no treatment; **test:** probiotic treatment; **6 mo.s:** 6 months without treatment; **placebo:** placebo treatment.

Fig. 1. Study follow-up period.

Table 1. Demographic and clinical description of the study population

	Group A: Healthy	Group B: Mucositis	Differences per group (test)
Age (mean ± SD)	63.6 ± 10.4	60.2 ± 7.4	NS (*t*)
Gender (% females)	59.1	58.3	NS (Chi^2)
Number of patients	22	12	
Number of implants	54	23	
Oral hygiene			
Never (%)	0	0	NS (MW)
1–2 times/d (%)	63.6	91.7	
3 times/d (%)	36.4	8.3	
Rehabilitated arch			
Upper (%)	31.8	33.3	NS (MW)
Lower (%)	45.5	41.7	
Both (%)	22.7	25.0	
Prosthesis			
Fixed (%)	31.8	16.7	NS (Chi^2)
OD Locator® (%)	45.5	33.3	NS (Chi^2)
OD Bar (%)	9.1	33.3	NS (Chi^2)
Combination of the above types (%)	13.5	16.7	

Chi^2, Chi^2 test; MW, *Z*-values for Mann–Whitney *U*-test; NS, not signficant; *t*, Student *t*-test.

(Proflow Incorporated) to record the collected amount of crevicular fluid in Periotron units (PU), followed by calculation of the concentration values of each sample using a standard curve; and (vii) placement of the sample in a filter-equipped Eppendorf tube (Millipore, Massachusetts, MA, USA), followed by storage at −80°C.

Analysis of cytokines

The peri-implant crevicular fluid was absorbed by each strip over 30 s of collection. Each sample was diluted in an Eppendorf tube with 200 mL of 50 mM phosphate buffer, pH 7.2, together with a pool of protease inhibitors (Roche Diagnostics GmbH, Mannheim, Germany) and 0.1 mM phenyl sulfonyl fluorate, and incubated for 2 h. The samples were centrifuged at 1000 *g* for 5 min, and the supernatant was stored at −80°C until use. IL-1β, IL-6 and IL-8 were evaluated in the supernatants stored at −80°C. The evaluation was performed using the Human Inflammation Cytometric Bead Array system (Becton Dickinson, BD Biosciences, San Diego, CA, USA) and cytofluorometry analysis (Becton Dickinson, BD Biosciences). The samples and positive controls (standard curve) were processed according to the instructions of the manufacturer, and the values for IL-1β, IL-6 and IL-8 were calculated and reported as pg/mL. Data were acquired with a fluorescence-activated cell sorter Microbiology Calibur flow cytometer (Becton Dickinson, Franklin Lakes, NJ, USA). Figure 2 specifies the cytokine battery used in the study (IL-1β, IL-6 and IL-8), together with the displacement plots according to cytokine concentration.

Treatment

Both the probiotic and placebo were presented in identical containers, except that one was coded "A" and the other "B". The probiotic formulation contained *Lactobacillus reuteri* Prodentis (GUM PerioBalance™; Sunstar, Etoy, Switzerland), with at least 200 million active units of the probiotic (strains ATCC PTA 5289 – 100 million, and DSM 17938 – 100 million). The placebo and probiotic tablets were identical, except for the absence of active drug substance in the former. After screening, participants received supragingival prophylaxis, with a rubber cup and abrasive

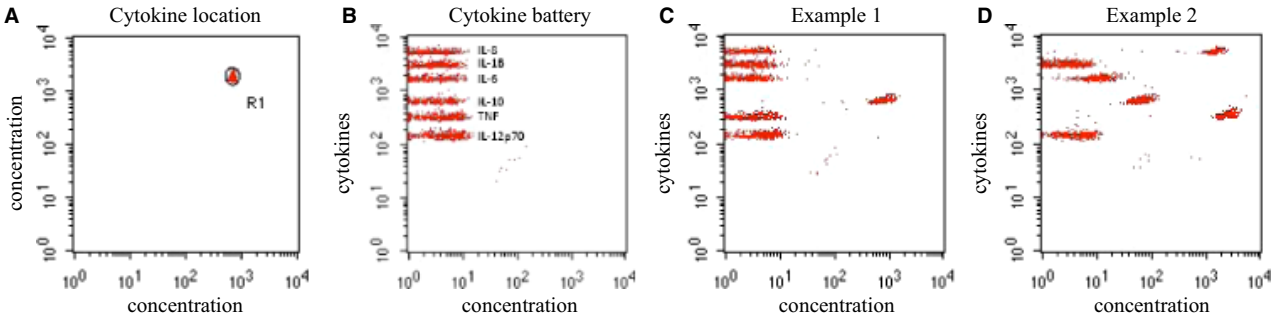


Fig. 2. Displacement plots according to cytokine concentration. (A) Cytokine concentration and location; (B) example of cytokine battery before the determination of concentration; (C) example 1 of determination of cytokine in sample 1 (IL-10 is displaced in the figure); (D) example 2 of determination of cytokine in sample 2 (IL-8, IL-6, IL-10; TNF-α is displaced in the figure; the greater the displacement to the right, the higher the contentration of the cytokine). IL, interleukin; TNF, tumor necrosis factor.

paste. One month later, they were scheduled for a baseline examination. On this visit, and following the determination of crevicular fluid volume, modified plaque index, probing depth, modified gingival index and concentrations of IL-1β, IL-6 and IL-8, the subjects were randomized to treatment with the study or placebo tablets. The dosage was one tablet every 24 h during 30 d (Fig. 1). One month later, when the patients returned for evaluation, they were required to bring the tablet containers with them. This allowed us to assess correct adherence to therapy. All patients started with probiotic treatment. The clinicians and examiners were blinded. After treatment with the probiotics, and following a 6 mo washout period, the same treatment protocol was repeated but using placebo. During follow-up after treatment in the washout period, the patients only received the same oral hygiene instructions as before.

Evaluation of the Hawthorne effect

The Hawthorne effect is defined as inadvertent behavioral change on the part of people implicated in a study as a consequence of the fact that they are participating in the study. These people become more careful in executing their assigned tasks simply because they have been included in the study and feel themselves to be under observation (34). Two measures were adopted to assess the presence or absence of this effect in the present study. The patients were appointed for recording of the study parameters, with no prescription of treatment, followed by repeat recording of the parameters 1 mo later to evaluate possible differences between the two time points.

Statistical analysis

In all bivariate analyses, the level of significance used was 5% ($\alpha = 0.05$). For a test such as the Wilcoxon test, used for follow-up of the clinical parameters and interleukin levels over time, with a level of significance of 5% and considering the detection of an effect size of 0.55, a sample size of 34 patients affords a statistical power of 0.821 (82.1%). Statistical power was calculated at implant level ($n = 77$), adjusting for within-subject correlation. Securing a statistical power of 80% would require 18 patients per group, i.e. 36 patients. The sample size in the present study was 34 patients. The Wilcoxon test was used due to the non-normal distribution of these parameters. Comparisons between the probiotic and placebo groups also imply the same statistical power, as the cross-over design of the study means that the patients act both as cases and as their own controls in the two phases of the study. These comparisons were performed by means of the chi-squared, Student t- and Mann–Whitney *U*-tests for categorical, normal and non-normal continuous parameters, respectively. The study was randomized with respect to initial treatment ("A" or "B") by the study supervisor (MPD) using the SPSS version 15.0 statistical package (SPSS Inc., Chicago, IL, USA) for MS Windows, which was likewise employed in all the statistical analyses.

Results

Patient data

A total of 34 patients were treated, with the evaluation of 77 dental implants (Fig. 3). Two groups were established: group A (22 patients and 54 implants without peri-implant disease) and group B (12 patients with peri-implant mucositis affecting one or more implants [23 implants with mucositis and seven implants without disease]). Table 1 reports the mean patient age, gender, oral hygiene, rehabilitated arch and type of prosthesis.

Before probiotic treatment, the clinical parameters were found increased in group B with respect to group A (Table 2). Before placebo, the differences were not so notorious – the plaque index being somewhat greater in group A than in group B. Following administration of the probiotic, the decreases in plaque, probing depth, gingival index and crevicular fluid were significantly greater than with placebo in both group A and group B.

In group A, the mean plaque index decreased 0.59 points with probiotic treatment and remained stable with

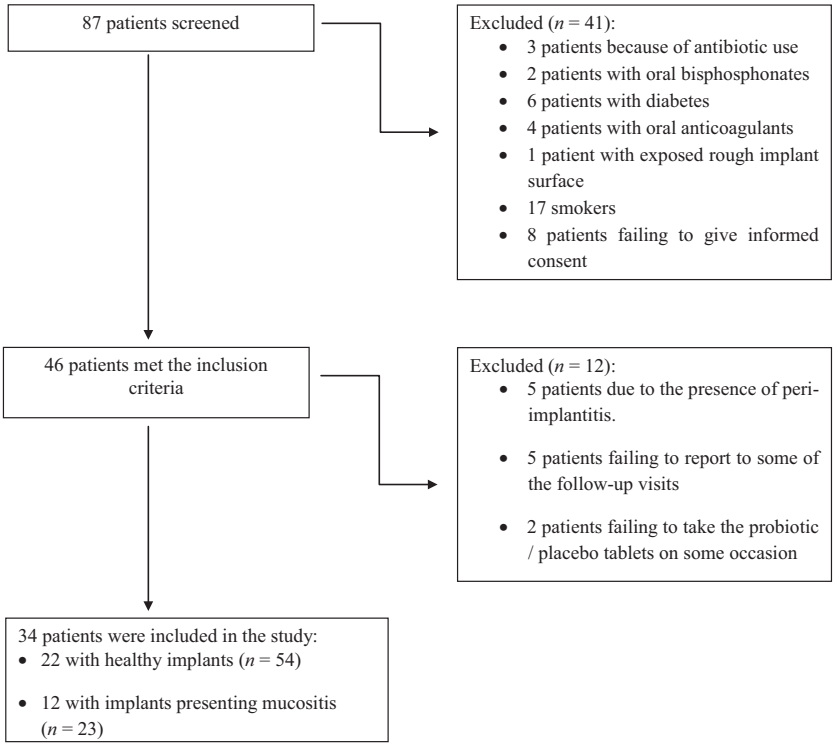


Fig. 3. Classification of the included and excluded patients following the CONSORT criteria for clinical studies.

placebo – the difference between the two groups being statistically significant ($p = 0.001$). In group B, the mean plaque index decreased 0.74 points with probiotic treatment and likewise remained stable with placebo ($p = 0.035$) (Table 2).

In group A, the mean PD decreased 0.16 mm after probiotic treatment but increased 0.27 mm after placebo ($p = 0.001$). In turn, in group B the mean decrease in PD was 1.09 mm, vs an increase of 0.18 mm after placebo ($p = 0.001$) (Table 2).

Crevicular fluid exhibited similar behavior. In the healthy implants (group A), a mean decrease of 39.9 units was observed after probiotic treatment, vs an increase of 18.7 units after placebo ($p < 0.001$). In the implants with mucositis (group B), the mean crevicular fluid reductions were 50.8 and 4.1 points following probiotic treatment and placebo, respectively ($p = 0.012$) (Table 2).

Likewise, in group A the mean gingival index decreased 0.37 points following probiotic treatment, and increased 0.44 points after placebo ($p < 0.001$). In group B, the mean gingival index was seen to stabilize after probiotic treatment (−0.09) and increased following placebo (0.48). This was the only case in which the difference in response failed to reach statistical significance ($p = 0.117$) (Table 2).

As regards the interleukins, before treatment with either the probiotic formulation or placebo, the concentrations of IL-6 and IL-8 were greater in group B than in group A (Table 3). The concentration of IL-1β was likewise greater in group B than in group A, but only before the probiotic treatment phase. Following probiotic administration, the decreases in IL-1β, IL-6 and IL-8 were greater than following placebo in both group A and group B – with differences that reached statistical significance in some cases.

In group A the mean IL-1β value decreased 7.7 units following the probiotic treatment phase and increased 4.0 units after placebo – the difference bordering statistical significance ($p = 0.059$) (Fig. 4 and Table 3). In group B the mean decrease was 29.9 units with the probiotic, vs only 7.5 with placebo, though the difference was not statistically significant ($p = 0.301$).

In group A, the mean IL-6 value decreased 0.04 units following probiotic treatment and increased 0.10 units in the placebo group. The variation can be regarded as similar in both groups ($p = 0.204$). However, in group B the cytokine level decreased 0.66 units on average after probiotic treatment vs only 0.15 units after placebo – the difference in this case being statistically significant ($p = 0.033$) (Fig. 5 and Table 3).

Lastly, in group A the mean IL-8 value decreased 122.5 units following probiotic treatment, which was in clear contrast to the evolution observed in the placebo group, with an increase of 69.9 units – the difference being statistically significant ($p < 0.001$). In group B, the mean decrease after probiotic treatment was 143.2 units, vs only 4.9 units after placebo – the difference once again being statistically significant ($p = 0.013$) (Fig. 6 and Table 3). No alterations or unintended effects were observed after the two treatments (probiotic and placebo).

Presence of mucositis

In group A, one implant developed mucositis during probiotic treatment. In the placebo phase, all the implants maintained their initial condition. In group B all the implants ($n = 23$) presented mucositis before probiotic treatment, while 17 implants (73.9%) were free of mucositis after such treatment. Following treatment with placebo, two initially affected implants (20%) were found to be free of mucositis.

Evaluation of the Hawthorne effect

Two consecutive, independent measurements were made (spaced 1 mo apart) in the absence of treatment of any kind, to identify possible alterations in the clinical and immunological parameters attributable to the fact

Table 2. Differences in clinical parameters according to study group and treatment

	Group A: healthy				Group B: mucositis			
	T1[a]	T2[a]	T2-T1	*p* (Wilc.)	T1	T2	T2–T1	*p* (Wilc.)
PI[a]								
Prob[a]	0.96 ± 1.03	0.37 ± 0.68	−0.59 ± 0.94	< 0.001	1.70 ± 1.22	0.96 ± 1.15	−0.74 ± 1.05	0.007
Plcb[a]	1.31 ± 1.18	1.31 ± 1.11	0.00 ± 0.82	1.000	1.09 ± 1.12	1.09 ± 1.12	0.00 ± 1.28	0.892
p (MW)	0.026	< 0.001	**0.001**		0.045	0.818	0.035	
PD[a]								
Prob[a]	2.72 ± 0.59	2.56 ± 1.03	−0.16 ± 0.84	0.037	3.55 ± 0.40	2.46 ± 0.92	−1.09 ± 0.90	< 0.001
Plcb[a]	2.55 ± 1.02	2.82 ± 1.16	0.27 ± 0.72	0.003	2.47 ± 0.79	2.65 ± 0.84	0.18 ± 0.65	0.161
p (MW)	0.018	0.027	0.001		< 0.001	0.113	0.001	
mGI[a]								
Prob[a]	0.63 ± 0.92	0.26 ± 0.56	−0.37 ± 0.73	0.001	1.39 ± 0.78	1.30 ± 0.70	−0.09 ± 1.08	0.557
Plcb[a]	0.46 ± 0.69	0.91 ± 0.90	0.44 ± 0.77	< 0.001	1.17 ± 0.49	1.65 ± 0.78	0.48 ± 0.79	0.013
p (MW)	0.163	< 0.001	< 0.001		0.248	0.183	0.117	
PICF [a]								
Prob[a]	91.7 ± 50.3 (87.5)	51.8 ± 38.9 (37.0)	−39.9 ± 55.4 (−33.0)	< 0.001	102.0 ± 49.0 (96.0)	51.2 ± 33.0 (45.0)	−50.8 ± 65.6 (−45.0)	0.005
Plcb[a]	62.8 ± 46.7 (51.0)	81.5 ± 50.3 (70.0)	18.7 ± 45.4 (6.0)	0.003	67.0 ± 43.7 (50.0)	62.9 ± 47.1 (52.0)	−4.1 ± 46.4 (0.0)	0.910
p (MW)	< 0.001	< 0.001	< 0.001		0.002	0.378	0.012	

mGI, modified gingival index; MW, *Z*-values for Mann–Whitney *U*-test; PD, probing depth; PI, plaque index; PICF, peri-implant crevicular fluid volume; Plcb, placebo; Prob, probiotic; T1, pre-treatment; T2, post-treatment; Wilc., *Z*-values for Wilcoxon test.

[a]Values presented as mean ± SD (median).

Bold value indicates statistically significant difference between T2 and T1 ($p = 0.001$).

of patient awareness of inclusion in the study (the Hawthorne effect). All the parameters showed similar values at both time points in the two groups, without significant differences between them. No interference due to the Hawthorne effect was thus observed (Table 4).

Discussion

While the literature contains studies of oral probiotics used to treat periodontal diseases, we have found no publications on the effect of probiotics upon peri-implant health. Koll-Klais *et al.* (7) observed 82% and 65% inhibition of the growth of *Porphyromonas gingivalis* and *Prevotella intermedia*, respectively, because of the administration of *Lactobacillus*, thereby improving the periodontal health of the patient. Krasse *et al.* (6) in turn reported a significant decrease in gingival index and in the amount of bacterial plaque in patients treated with *Lactobacillus reuteri* with respect to a control group administered placebo. The authors concluded that this probiotic is effective in reducing gingivitis and bacterial plaque accumulation in patients with moderate to severe gingivitis. Harini and Anegundi (9) evaluated the effects of probiotic and chlorhexidine rinses upon periodontal health. These investigators observed a decrease in plaque accumulation and in the modified gingival index; the reductions being comparatively greater with the probiotic rinse. After administering the probiotic *Lactobacillus salivarius* three times a day over 8 wk in 66 patients, Shimauchi *et al.* (35) recorded a significant decrease in the bacterial plaque index, with no differences in probing depth. The non-placebo-controlled study published by Della Riccia *et al.* (36) found the administration of *Lactobacillus brevis* to reduce the bacterial plaque index after 4 d of treatment. Vicario *et al.* (37) conducted a randomized, double-blind study of the effect of *Lactobacillus reuteri* in healthy non-smokers with early stage to moderate periodontitis, and recorded improvement of all the studied clinical parameters (plaque index,

Table 3. Differences in immunological parameters according to study group and treatment

	Group A: healthy				Group B: mucositis			
	T1[a]	T2[a]	T2–T1	*p* (Wilc.)	T1	T2	T2–T1	*p* (Wilc.)
IL-1β[a]								
Prob[a]	21.2 ± 24.2 (8.9)	13.5 ± 24.8 (4.7)	−7.7 ± 28.2 (−3.0)	0.006	42.5 ± 80.6 (15.7)	12.6 ± 15.4 (5.3)	−29.9 ± 81.6 (−6.9)	0.019
Plcb[a]	22.9 ± 30.1 (12.1)	26.9 ± 40.2 (15.5)	4.0 ± 45.0 (−3.7)	0.555	21.9 ± 32.3 (10.1)	14.5 ± 18.9 (6.8)	−7.5 ± 36.9 (−4.7)	0.128
p (MW)	0.881	0.001	0.059		0.412	0.563	0.301	
IL-6[a]								
Prob[a]	0.53 ± 0.63 (0.0)	0.49 ± 0.60 (0.0)	−0.04 ± 0.87 (0.0)	0.514	0.99 ± 0.49 (1.17)	0.33 ± 0.53 (0.0)	−0.66 ± 0.70 (−1.07)	0.002
Plcb[a]	1.07 ± 0.36 (1.10)	1.17 ± 0.38 (1.19)	0.10 ± 0.55 (0.03)	0.026	1.19 ± 0.29 (1.23)	1.04 ± 0.58 (1.23)	−0.15 ± 0.68 (0.05)	0.879
p (MW)	< 0.001	< 0.001	0.204		0.140	0.002	0.033	
IL-8[a]								
Prob[a]	193.9 ± 164.8 (157.7)	71.4 ± 88.0 (37.7)	−122.5 ± 178.0 (−100.7)	< 0.001	243.2 ± 273.0 (146.3)	100.1 ± 93.4 (71.3)	−143.2 ± 270.7 (−88.4)	0.002
Plcb[a]	124.2 ± 158.7 (106.3)	194.1 ± 245.8 (127.6)	69.9 ± 300.0 (46.7)	0.007	178.9 ± 183.0 (124.2)	173.9 ± 108.3 (177.2)	−4.9 ± 223.5 (41.9)	0.670
p (MW)	0.002	< 0.001	< 0.001		0.153	0.026	0.013	

IL, interleukin; MW, *Z*-values for Mann–Whitney *U*-test; Plcb, placebo; Prob, probiotic; T1, pre-treatment; T2, post-treatment; Wilc., *Z*-values for Wilcoxon test.
[a]Values presented as mean ± SD (median).

bleeding upon probing and probing depth) after 30 d of treatment vs placebo. The present study has obtained similar results, since the application of *Lactobacillus reuteri* Prodentis led to a statistically significant decrease in crevicular fluid volume, modified plaque index, probing depth and in the concentration of IL-1β and IL-8 in both groups (A and B). In turn, a significant reduction of the modified gingival index was recorded in group A, with a non-significant reduction in group B. Lastly, a significant reduction in IL-6 concentration was observed in group B, while in group A the decrease failed to reach statistical significance. Twetman *et al*. (8), after treatment with probiotics, also recorded a decrease in bleeding and in the concentration of TNF-α and IL-8 vs placebo.

These findings of the present study differ from those of other studies found in the literature, where non-significant results were obtained. As an example, Sinkiewicz *et al*. (38), administered the probiotic *Lactobacillus reuteri* or the corresponding placebo during 12 wk to 23 patients without periodontal disease, and observed no statistically significant differences after treatment. Staab *et al*. (39) in turn conducted a study with the probiotic *Lactobacillus casei*. The patients ($n = 50$) were divided into two groups: a test group administered milk with the probiotic once a day, and a control group. After 8 wk, no change was noted in the bacterial plaque index. Iniesta *et al*. (40), after administering *Lactobacillus reuteri* to a group of 40 patients with gingivitis, recorded no significant clinical differences, though significant microbiological modifications were observed. Regarding the treatment of mucositis, Renvert *et al*. (41) conducted a review with the inclusion of five articles. These studies evaluated the treatment of mucositis using chlorhexidine rinses (42,43), Listerine® (Pfizer, Morris Plains, NJ, USA) (44), submucosal tetracycline fibers (45), or phosphoric acid gel (46). In all cases, the elimination of bacterial plaque was seen as the cause of a decrease in mucositis, pocket depth and bleeding upon prob-

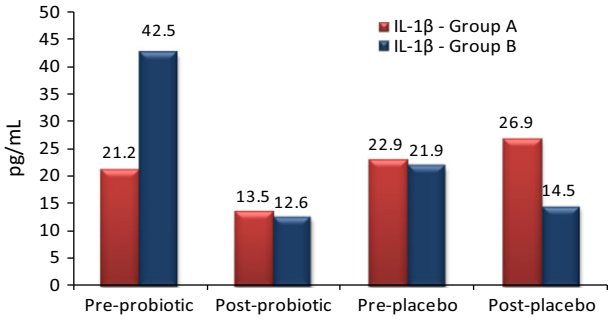


Fig. 4. Concentration of IL-1β by groups and treatment. IL, interleukin.

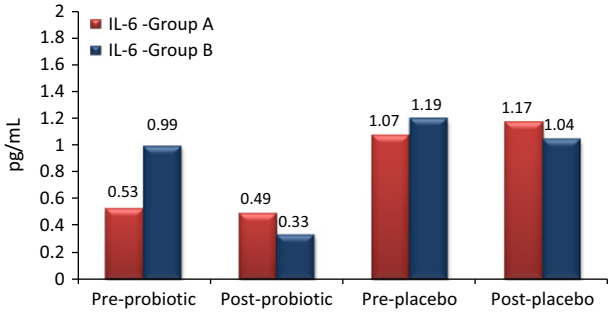


Fig. 5. Concentration of IL-6 by groups and treatment. IL, interleukin.

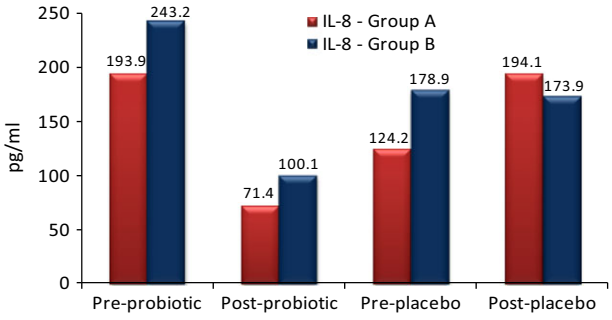


Fig. 6. Concentration of IL-8 by groups and treatment. IL, interleukin.

ing. None of the mentioned treatments was found to be superior to the rest. In contrast, in the present study, administration of the probiotic *Lactobacillus reuteri* Prodentis improved the peri-implant health in both study groups – this being the reason for proposing probiotics as a treatment alternative. We chose a dose of one tablet every 24 h during 30 d, since such dosing is consistent with the recommendations of the manufacturer and with the dosage used in other important studies (6,37,40). In a recent study, Maekawa and Hajishengallis (47) concluded that the probiotic *Lactobacillus brevis CD2* could inhibit periodontitis through modulating effects on the host response and the periodontal microbiota.

The results of our study cannot be compared with those of other studies involving the application of probiotics in dental implants. There is insufficient evidence to support the efficacy of probiotics in treating periodontal disease (48). This situation constitutes a limitation, and further studies are needed to compare the effects of probiotics in the periodontal and peri-implant tissues. In addition, in this context, we consider it

Table 4. Mean values corresponding to the clinical parameters and concentrations of IL-1β, IL-6 and IL-8, assessing the Hawthorne effect according to the study groups

	PICF		PI		PD		mGI		IL-1β		IL-6		IL-8	
	Group A[a]	Group B[a]	Group A	Group B	Group A	Group B	Group A	Group B	Group A	Group B	Group A	Group B	Group A	Group B
1st	53.9 ± 39.5	50.6 ± 30.8	0.50 ± 0.78	1.63 ± 1.12	2.81 ± 1.11	2.33 ± 0.90	0.43 ± 0.68	1.21 ± 1.32	11.6 ± 14.3 (5.8)	28.4 ± 19.2 (11.6)	0.39 ± 0.41 (0.0)	1.23 ± 0.22 (1.17)	128.2 ± 112.5 (102.6)	161.8 ± 107.0 (112.4)
2nd	53.8 ± 32.3	58.7 ± 38.6	0.63 ± 0.81	1.68 ± 1.16	2.85 ± 1.03	2.44 ± 0.77	0.57 ± 0.83	1.11 ± 1.33	13.2 ± 16.6 (6.2)	27.9 ± 20.1 (11.1)	0.43 ± 0.33 (0.0)	1.11 ± 0.38 (1.21)	131.6 ± 118.1 (112.1)	166.4 ± 101.1 (152.5)
p	0.931	0.305	0.206	0.317	0.360	0.096	0.102	0.705	0.551	0.867	0.511	0.766	0.822	0.653

Mean ± SD (median).
[a]IL, interleukin; mGI, modified gingival index; *p*, *p* value for Wilcoxon test (*Z*-value); PD, probing depth; PI, plaque index; PICF, peri-implant crevicular fluid volume.

important to note that following oral probiotic administration, peri-implant health was improved at subclinical and clinical level, with a decrease in the concentrations of inflammatory mediators.

Study limitations

One of the limitations of the present study is its cross-over design and statistical power. In effect, although the power was sufficient (82.1%), a larger study sample would have been desirable. Another limitation is the fact that there are no other similar studies allowing comparisons of the effect of probiotics in dental implants.

Conclusions

After treatment with the probiotic *Lactobacillus reuteri* in patients with implants presenting mucositis, the clinical parameters improved, and the cytokine levels decreased – in contraposition to the observations in the placebo group. A decrease in the clinical and immunological parameters was also recorded in the group of patients with implants without peri-implant disease. Probiotic administration may be regarded as a good alternative for both the treatment of peri-implant mucositis and its prevention, as it improved clinical parameters in the healthy individuals. Further studies involving larger patient samples are needed regarding the effects of probiotics upon peri-implant health.

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