**Clinical effects of probiotics containing Bacillus species on gingivitis: a pilot randomized controlled trial.**

For figures, tables and references we refer the reader to the original paper.

Abstract

Background and Objective

*Lactobacillus* spp. and bifidobacteria are the most frequently used probiotics in oral health research. However, although probiotic effects have been suggested for other genera, such as bacilli, no trials are available to describe the effect of bacilli probiotics on gingivitis in humans. The aim of the present study was to evaluate the clinical effects of a bacilli-containing toothpaste, a mouthrinse and a toothbrush cleaner versus a placebo in patients with generalized gingivitis.

Material and Methods

In this double-blind placebo-controlled randomized clinical trial, nonsmoking, systemically healthy patients with generalized gingivitis were included. They used a placebo or an experimental probiotic *Bacillus subtilis*-, *Bacillus megaterium*- and *Bacillus pumulus*-containing toothpaste, mouthrinse and toothbrush cleaner for 8 wk. Primary outcome measures of interest were plaque and gingivitis index, and the secondary outcome measures were pocket probing depth and bleeding on probing.

Results

Twenty male and 20 female patients were randomized over the two groups. All participants could be included in the final analysis. Although plaque and gingivitis indices were significantly reduced after 8 wk, no intergroup differences could be found at any time point. Also, for the secondary outcome measure, intragroup but no intergroup differences could be detected. No harm or unintended effects were reported by the patients after using the study products.

Conclusions

This study did not show any statistically significant differences between a placebo and a bacilli-containing toothpaste, mouthrinse and toothbrush cleaner on gingivitis parameters.

The administration of microorganisms to improve human health had already been promoted by the Nobel prizewinning scientist Eli Metchnikoff at the beginning of the 20th century [[1](http://onlinelibrary.wiley.com/doi/10.1111/jre.12415/full#jre12415-bib-0001)]. However, it took several decades before this subject became a topic of interest in medicine. Currently, these microorganisms are called “probiotics” and are defined as “living microorganisms which, when administered in adequate amounts, confer a health benefit for the host” (<http://who.int/foodsafety/fs_management/en/probiotic_guidelines.pdf>). Over the past decade, probiotics have been extensively investigated from the perspective of oral health. It has been shown that, during use, probiotics have the capacity to reduce mutans streptococci counts in saliva and/or plaque [[2, 3](http://onlinelibrary.wiley.com/doi/10.1111/jre.12415/full#jre12415-bib-0002)], that they have a positive influence on bad breath [[4-6](http://onlinelibrary.wiley.com/doi/10.1111/jre.12415/full#jre12415-bib-0004)] and that some can improve the results of scaling and root planing in patients with periodontitis [[7-10](http://onlinelibrary.wiley.com/doi/10.1111/jre.12415/full#jre12415-bib-0007)]. A positive effect of probiotic use on gingival indices [[11-14](http://onlinelibrary.wiley.com/doi/10.1111/jre.12415/full#jre12415-bib-0011)] and on plaque accumulation have been shown repeatedly [[11, 12, 15](http://onlinelibrary.wiley.com/doi/10.1111/jre.12415/full#jre12415-bib-0011)]. To date, the most widespread mechanical tool to control plaque and prevent gingival inflammation and subsequent attachment loss is still toothbrushing [[16](http://onlinelibrary.wiley.com/doi/10.1111/jre.12415/full#jre12415-bib-0016)]. However, most individuals do not adequately control plaque accumulation and gingivitis is still highly prevalent [[17, 18](http://onlinelibrary.wiley.com/doi/10.1111/jre.12415/full#jre12415-bib-0017)]. The use of probiotics to combat gingival inflammation has already been described [[11, 13, 15, 19-23](http://onlinelibrary.wiley.com/doi/10.1111/jre.12415/full#jre12415-bib-0011)]. These studies showed positive effects of different *Lactobacillus* strains [[11, 13, 15, 19-23](http://onlinelibrary.wiley.com/doi/10.1111/jre.12415/full#jre12415-bib-0011)]. In oral health research, next to *Lactobacillus* species, bifidobacteria are amongst the most frequently used probiotics [[3, 24](http://onlinelibrary.wiley.com/doi/10.1111/jre.12415/full#jre12415-bib-0003)]. However, in the oral cavity, probiotic effects have been suggested for other genera, such as bacilli. It has been repeatedly shown that bacilli-containing probiotics have a positive effect (less tissue breakdown) on ligature-induced periodontitis in rats [[25-27](http://onlinelibrary.wiley.com/doi/10.1111/jre.12415/full#jre12415-bib-0025)]. In humans, a mouthrinse containing *Bacillus subtilis* was significantly more effective in reducing periodontal pathogens compared with a mouthrinse containing benzethonium chloride as adjunctive to initial periodontal treatment [[28](http://onlinelibrary.wiley.com/doi/10.1111/jre.12415/full#jre12415-bib-0028)]. However, to the best of our knowledge, at present no trials are available describing the effect of bacilli-containing probiotics on gingivitis. As probiotic effects are strain-, dosage- and mode of application-dependent [[24](http://onlinelibrary.wiley.com/doi/10.1111/jre.12415/full#jre12415-bib-0024)], the aim of this pilot study was to evaluate the use of an experimental probiotic toothpaste, mouthrinse and toothbrush cleaner containing *Bacillus subtilis, Bacillus megaterium* and *Bacillus pumulus* spores versus a placebo in patients with generalized gingivitis.

Material and methods

This double-blind, placebo-controlled, randomized (1 : 1 ratio) controlled clinical trial with two parallel arms was approved by the local Ethics Committee of Cukurova University (Adana, Turkey), with the number CUTFEK-March3, 2014-29-14. The trial is registered at clinicaltrials.gov with identifier: NCT02597192. Dentate patients referred for periodontal treatment at the Department of Periodontology, Cukurova University Faculty of Dentistry (Adana, Turkey) were screened for the study. Patients were included if: (i) they were diagnosed with plaque-induced gingivitis [i.e. bleeding on gentle probing at >30% of sites examined and a gingival index (GI) [[29](http://onlinelibrary.wiley.com/doi/10.1111/jre.12415/full#jre12415-bib-0029)] of at least 1 at > 60% of sites examined]; (ii) had a plaque index (PI) of ≥ 2 according to the modified Quigley & Hein index [[30](http://onlinelibrary.wiley.com/doi/10.1111/jre.12415/full#jre12415-bib-0030)]; (iii) were 18–60 years old; and (iv) had at least 20 natural teeth. The exclusion criteria were: (i) pocket probing depth or clinical attachment loss of > 4 mm; (ii) the presence of hematologic disorders or other systemic illness; (iii) pregnancy and breastfeeding; (iv) current orthodontic treatment; (v) history of periodontal therapy; (vi) use of antibiotics or anti-inflammatory medication within the preceding 6 months; and (vii) smoking.

Examiner calibration

One blinded trained examiner (O.O.) performed all clinical measurements using a periodontal probe (PCP-UNC15; Hu-Friedy, Chicago, IL, USA). Calibration of the examiner was conducted before the study to ensure the intra-examiner reproducibility of the clinical measurements. In brief, the examiner evaluated, with a 3-h intervening period between subjects, the clinical parameters of six subjects who were not involved in the study and had gingivitis. The intraclass test was used to determine the intra-examiner reproducibility for GI and bleeding on probing scores. The examiner achieved intra-class correlation values of 0.96.

Outcome variables of interest

All variables were recorded when the patients were recruited (−3 wk), baseline (BL) and after 8 wk (8 wk) of use of the probiotic or placebo toothpaste, mouthrinse and toothbrush cleaner.

Primary outcome measures

The primary outcome measures were PI and GI. The PI was measured after discoloration with Mira-2-ton disclosing solution at the vestibular surfaces of the teeth according to the Turesky modification of the Quigley & Hein index, as follows: 0, no plaque; 1, separate spots of plaque at the cervical margin of the tooth; 2, a thin, continuous band of plaque at the cervical margin; 3, a band of plaque wider than 1 mm but covering less than one-third of the tooth; 4, plaque covering more than one-third and less than two-thirds of the crown; and 5, plaque covering more than two-thirds of the crown [[30, 31](http://onlinelibrary.wiley.com/doi/10.1111/jre.12415/full#jre12415-bib-0030)]. The mean PI was obtained by dividing the sum of all plaque scores by the total number of scored surfaces examined.

The GI was measured at six sites per tooth, according to Loë & Silness [[29](http://onlinelibrary.wiley.com/doi/10.1111/jre.12415/full#jre12415-bib-0029)], as follows: 0, normal gingiva; 1, mild inflammation with slight change in color, mild alteration of gingival surface structure and no bleeding on probing; 2, moderate inflammation with edema, redness, swelling and bleeding on probing; and 3, severe inflammation with marked edema and redness, ulceration and tendency to bleed spontaneously. The mean GI was calculated by dividing the sum of all scores by the total number of surfaces examined. Patients were also grouped, depending on the severity of gingivitis, with a score of 0.1–1.0 representing mild inflammation, a score of 1.1–2.0 representing moderate inflammation and a score of 2.1–3.0 representing severe inflammation.

Secondary outcome measures

The secondary outcome measures were pocket probing depth and bleeding on probing. These were measured using a North Carolina periodontal probe (Hu-Friedy) at six sites per tooth. Furthermore, the tongue coating was scored according to Winkel [[32](http://onlinelibrary.wiley.com/doi/10.1111/jre.12415/full#jre12415-bib-0032)] by dividing the tongue into three equal parts, from anterior to posterior. The tongue coating in each third was scored as follows: 0, no coating; 1, light coating; and 2, severe coating. These scores were obtained chairside, and clinical photographs of the tongue were made to verify the initial assessments.

Randomization

Randomization of the patients was carried out by block randomization (version 2.7.3; Stats-Direct, Cheshire, UK) at the beginning of the study. The coded products were given to the examiner (O.O.) by the study coordinator (M.C.H.) at the baseline visits. Except for the study coordinator (M.C.H.), all study personnel and the patients were blinded regarded allocation to the study test and control groups. Before sending the data to the statistician, the code was broken to group the patients.

Treatment protocol

Patients who met the inclusion criteria were invited to participate in this study and, upon agreement, to sign an informed consent form. At this recruitment visit (−3 wk), all patients received supragingival scaling and/or oral prophylaxis and entered a washout period for 3 wk. Then, the patients were asked to attend for baseline recordings to be made and they were told not to perform any oral hygiene (including chewing gum) for 8 h before this visit. This recommendation also applied to the follow-up examination. Baseline examination consisted of scoring of the tongue coating, GI, bleeding upon probing and pocket probing depth. After recording the clinical data, a coded package containing one toothbrush (Signal white power, medium: unilever ltd. Istanbul, Turkey), one probiotic toothpaste, two probiotic mouthrinses and one box of a probiotic toothbrush cleaner (Chrisal, Lommel, Belgium) were given to the patients. All of these products were experimental formulations that contained 5 × 107 colony-forming units of *B. subtilis, B. megaterium* and *B. pumulus* spores. Half of the group received the probiotic products and the other half received placebo products that were identical in shape, texture, taste and smell to the probiotic products. The patients were told that during the test period they should only use the material provided by the manufacturer for the test, including the toothbrush. Patients were asked to refrain from all other unassigned forms of oral hygiene, including nonstudy toothbrushes or toothpastes, interdental cleaning aids, chewing gum or oral rinses, during the study.

Subsequently they received the following instructions:

* Brush your teeth two times a day using the toothpaste.
* For preparing the experimental toothbrush cleaner: add 1 ampule of the experimental toothbrush cleaner in a glass and add mineral water till the glass is halfway full (100 mL).
* After brushing, rinse the toothbrush well and place the toothbrush head in a glass with the experimental toothbrush cleaner.
* The glass with the experimental toothbrush cleaner should be replaced every week.
* When you brush your teeth the next time, do not rinse the toothbrush, just pick it out of the glass add the toothpaste and start brushing.
* Every evening, before going to sleep, fill one cap with the experimental mouthrinse, rinse your mouth with it for at least 1 min and spit it out.
* The mouthrinse should not be diluted, just fill the cap with pure product.

After 8 wk of the usage of these products, the patients were asked to return to the clinic for a follow-up visit. At this appointment, the same measurements as taken at baseline were made.

Compliance and adverse effects

Weekly telephone calls were made throughout the study to evaluate and increase patient compliance. Additionally, the patients returned the test products at the 8-wk visit to check for compliance. At each control visit, the examiner (O.O.) questioned the patient in relation to general health changes, use of anti-inflammatory drugs, use of mouthrinses, compliance of the use of probiotic products and any adverse events that the patients might have noticed.

Statistical analysis and sample size analysis

Data were analyzed using S-Plus 8.0 for Linux (Tibco, Palo Alto, CA, USA). The subject was the unit in all statistical tests performed. An ANOVA model was built to compare days per treatment and treatment per days. Normality and equality of variance were assessed using a normal quantile plot and a residual plot, respectively. For the ranking, depending on the severity of gingivitis a generalized linear model was applied to model the proportion of mild (with respect to moderate or severe) gingivitis on the one hand and mild or moderate (with respect to severe) gingivitis on the other hand. For each variable investigated, corrections for simultaneous hypothesis testing were performed according to Sidak. For all measurements, statistical significance was set as *p* ≤ 0.05, and when statistical significance was *p* ≤ 0.1 to > 0.05 this was described as “tendency”.

As there were no studies available using similar probiotics or a similar mode of application, a sample size analysis was not performed. This study should be considered as a pilot trial.

Results

In this study, 21 female and 19 male patients, 18–31 years of age, were included. All patients recruited completed the study. Recruitment of patients and clinical measurements were carried out between June and December, 2014. Figure [1](http://onlinelibrary.wiley.com/doi/10.1111/jre.12415/full#jre12415-fig-0001) displays the flow chart of the study and Fig. [2](http://onlinelibrary.wiley.com/doi/10.1111/jre.12415/full#jre12415-fig-0002) shows the study course. More detailed patient demographic characteristics are shown in Table [1](http://onlinelibrary.wiley.com/doi/10.1111/jre.12415/full#jre12415-tbl-0001). No harm or unintended effects were reported by the patients, and these were not noticed by the investigators at the 8-wk visit. Based on the study products returned and on questioning the patients, there were no compliance problems noted.

Figure 1.

Figure 2.

Study course. −3w, 3 wk before the study started; 8w, 8 wk after usage of the study products; BL, baseline.

Table 1. Demographic characteristics

Primary outcome measure

Table [2](http://onlinelibrary.wiley.com/doi/10.1111/jre.12415/full#jre12415-tbl-0002) displays the primary and secondary outcome measures and indicates the statistically significant inter- and intragroup differences. Regarding PI and GI, no statistically significant differences could be found between the groups at baseline, and an intergroup difference at recruitment or after 8 wk of the use of the study products was also not found. Moreover, no differences between the groups were found when the intragroup differences (delta) between the different time points were analyzed. By contrast, the PI and GI were significantly reduced in the control and probiotic groups between the baseline visit and after the use of the study product (*p* < 0.001 for all conditions and groups), between the recruitment visit and the baseline visit (*p* < 0.001 for all conditions and groups) and between the recruitment visit and the 8-wk visit (*p* < 0.001 for all conditions and groups). Also, when categorizing the patients into three groups based on their GI scores (mild, moderate and severe), no intergroup differences could be found (Fig. [3](http://onlinelibrary.wiley.com/doi/10.1111/jre.12415/full#jre12415-fig-0003)).

Table 2. Overview of all parameters examined

Figure 3.

Distribution of gingival index (GI) scores 3 wk before the study started (−3w), baseline (BL) and 8 wk (8w) after usage of the study products. C, control; P, probiotic.

Secondary outcome measures

When verifying the tongue coating scores with the aid of the clinical photographs, no changes were made from the chairside-assigned scores. No intergroup differences could be detected for tongue coating and pocket probing depth when comparing both groups at recruitment, baseline and 8 wk after the usage of the study products, or when looking at the intergroup comparisons of change (delta) of pocket probing depth or tongue coating at different time points. By contrast, statistically significant intragroup differences (less tongue coating and shallower pockets) could be found for tongue coating and pocket probing depth for both the control and the probiotic group after the 8-wk usage of the study products compared with baseline (respectively for the control group: *p* = 0.003 and *p* = 0.016, for the probiotic group: *p* = 0.001 and *p* = 0.009), between the recruitment and the 8-wk visit (*p* < 0.001 for all conditions and groups), and between the recruitment visit and the baseline visit (respectively for the control group: *p* = 0.001 and *p* < 0.001, for the probiotic group: *p* = 0.034 and *p* < 0.001).

Regarding bleeding on probing, no intergroup differences could be detected at recruitment, baseline or at the end of the study. Also, no statistically significant intergroup differences in change in bleeding on probing (delta bleeding on probing) were detected at the different time points. For bleeding on probing, statistically significant different intragroup differences were found between baseline and the 8-wk visit (control group: *p* < 0.001, probiotic group: *p* < 0.001), between recruitment and the 8-wk visit (control group: *p* < 0.001, probiotic group: *p* < 0.001) and between the recruitment visit and the baseline visit (control group: *p* < 0.001, probiotic group: *p* < 0.001).

Discussion

This pilot study compared, in patients with generalized gingivitis, the clinical effects of an experimental probiotic toothpaste, mouthrinse and toothbrush cleaner containing *B. subtilis, B. megaterium* and *B. pumulus* spores versus a placebo. Three different modes of application were used in order to introduce as much probiotic as possible into the oral cavity. Concerning the primary outcome measures, namely PI and GI, no intergroup differences could be found at any time point (recruitment, baseline and after 8 wk of use of the study products). Intragroup differences were noted when comparing the 8-wk results with the baseline/recruitment data and the baseline data with the recruitment data. This applied for both the control group and the probiotic group. For the secondary outcome measures (pocket probing depth, bleeding on probing and tongue coating) no statistically significant intergroup differences could be found. Consequently, this study could not show any benefit of the usage of probiotic oral-hygiene products containing *B. subtilis, B. megaterium* and *B. pumulus* spores over placebo products in patients with generalized gingivitis.

It is believed that probiotic products, in accordance with antiseptic products, only exert their benefits when the acquired biofilm is first removed [[7, 33](http://onlinelibrary.wiley.com/doi/10.1111/jre.12415/full#jre12415-bib-0007)]. However, as the aim of this study was to investigate the effect on patients with gingivitis, it was attempted to mimic this clinical situation. It was decided to remove the supragingival calculus before the start of the study because supragingival calculus has a considerable influence on the gingivitis measurements. A possible drawback is the rather complicated protocol for using the toothbrush cleaner: the study subjects had to prepare the toothbrush cleaner themselves and remember to refresh it every week. Therefore, people could have easily forgotten to refresh the toothbrush cleaner every week or not made optimal dilutions. However, good compliance was reported based on the products returned. Furthermore, the only information available from the company was the total amount of bacteria in each product, so it is not clear what the concentration was of each strain separately. Finally, it was difficult to perform a meaningful power analysis before the start of the study because no previous randomized controlled trials on this study product were available. Therefore, the study was considered to be a pilot study in nature. However, with the observed differences, 253 and 923 subjects in each group are needed to find a statistically significant difference for, respectively, PI and GI with α = 0.05 and power of 0.8.

*Bacillus* spp., considered as allochthonous microorganisms, are spore-forming microorganisms that are extensively used in humans as dietary supplements [[34, 35](http://onlinelibrary.wiley.com/doi/10.1111/jre.12415/full#jre12415-bib-0034)]. However, the use of these microorganisms in the dental field is still uncommon. A previous study showed that in healthy Indian children a 14-d administration of *Bacillus coagulans* led to a statistically significant reduction in salivary mutans streptococci counts [[36](http://onlinelibrary.wiley.com/doi/10.1111/jre.12415/full#jre12415-bib-0036)]. Additionally, in patients with periodontitis, a mouthrinse containing Extraction 300E (E-300; AHC Co., Gunma, Japan), prepared from the supernatant of culture medium of *B. subtilis*, significantly reduced the number of periodontal pathogens when compared with rinsing with benzethonium chloride as adjuncts to initial periodontal treatment [[28](http://onlinelibrary.wiley.com/doi/10.1111/jre.12415/full#jre12415-bib-0028)]. Three animal studies of the same research group showed positive effects of adding *Bacillus* species to the drinking water of rats with ligature-induced periodontitis. In unstressed rats with ligature-induced periodontitis, *B. subtilis* (CH201) reduced the attachment loss and the bone loss [[25, 26](http://onlinelibrary.wiley.com/doi/10.1111/jre.12415/full#jre12415-bib-0025)]. More recently, a protective effect against bone loss of a mixture containing *B. subtilis* and *Bacillus licheniformis* was shown in rats with ligature-induced periodontitis [[27](http://onlinelibrary.wiley.com/doi/10.1111/jre.12415/full#jre12415-bib-0027)]. However, the present study is, to our knowledge, the first study to investigate the effect of bacilli probiotics on gingivitis in humans. When looking at the results of studies reporting probiotic use in patients with gingivitis, Krasse *et al*. were the only researchers to report, in one (of two) *Lactobacillus reuteri* groups, a significantly improved GI compared with the placebo group [[11](http://onlinelibrary.wiley.com/doi/10.1111/jre.12415/full#jre12415-bib-0011)]. The PI was already significantly different at baseline and the intergroup differences were not reported at follow up. Shimauchi *et al*. could not find statistically significant differences for PI, GI, bleeding on probing and pocket probing depth between groups in terms of delta change when using *Lactobacillus salivarius* WB21 [[19](http://onlinelibrary.wiley.com/doi/10.1111/jre.12415/full#jre12415-bib-0019)]. Iniesta *et al*. could not find intergroup differences in PI and GI when using *L. reuteri* (DSM-17938 and ATCC PTA D289) [[22](http://onlinelibrary.wiley.com/doi/10.1111/jre.12415/full#jre12415-bib-0022)]. Only one other study investigated the effect of probiotics on tongue coating. Iwamoto *et al*. did not find statistical differences in tongue coating after 4 wk of use of *L. salivarius* WB21 in patients with halitosis [[5](http://onlinelibrary.wiley.com/doi/10.1111/jre.12415/full#jre12415-bib-0005)]. This is in accordance with the results of the experiment in the present study in which no effect of probiotic usage in patients with gingivitis was found on tongue coating.

On reflection, a significant improvement of the gingivitis measurements was detected in both the probiotic group and the placebo group. The intragroup differences between the recruitment visit and the baseline visit are probably a result of the temporary positive effect of the professional cleaning at the recruitment visit and the knowledge of the patients that they are being followed up in a study. This first aspect is also mentioned by Krasse *et al*. [[11](http://onlinelibrary.wiley.com/doi/10.1111/jre.12415/full#jre12415-bib-0011)]. They write that in their clinical experience the effect of a professional prophylaxis lasts for 1–3 wk and then plaque and gingivitis start to reappear. But, from these data, it seems that this effect not only lasts for 3 wk (until baseline), but also up to 11 wk (baseline + 8 wk of the study product usage). Additionally, part of these intragroup differences are probably also a result of the Hawthorne effect [[37](http://onlinelibrary.wiley.com/doi/10.1111/jre.12415/full#jre12415-bib-0037)]. The patients may have started brushing more effectively or more regularly, although they were asked not to modify their oral hygiene regimen because they knew they were being monitored.

A possible reason why this product did not show any advantages compared with the placebo product might be a low concentration of the probiotic. All three products contained 5 × 107 spores of *B. subtilis,* of *B. megaterium* and of *B. pumulus*. In contrast, the *Bacillus* spp. products used in the animal study of Messora contained 1.5 × 108 colony-forming units/mL and each of the rats was given 10 mL of drinking water/day, equivalent to 1.5 × 109 colony-forming units/d. In dentistry, there are still no guidelines of the concentration needed to have a positive effect on periodontal diseases or caries. However, to prevent antibiotic-associated diarrhea, a concentration of 5–40 × 109 colony-forming units/d of *Lactobacillus rhamnosus* or *Saccharomyces boulardii* is suggested [[38](http://onlinelibrary.wiley.com/doi/10.1111/jre.12415/full#jre12415-bib-0038)]. Another possible reason is that in the present study, bacilli spores are used, whereas in the previous periodontal studies other forms (no spores) were used [[25-28](http://onlinelibrary.wiley.com/doi/10.1111/jre.12415/full#jre12415-bib-0025)]. As no attempt was made to identify the probiotic strain intra-orally, it is not known whether the spores germinated. However, at this time it is not clear if the probiotic effect of bacilli spores is dependent on the germinated spores or if also the spore itself has a systemic effect.

Given the positive effects of bacilli-containing probiotics on periodontal disease in previous studies, bacilli-containing probiotics should still be considered as an option to combat oral diseases. However, more profound knowledge is necessary regarding the spore formation and germination of *Bacillus* species. Additionally, the ideal vehicle, concentration and application time should be investigated.

Conclusion

In conclusion, this study could not show any statistically significant differences between a group of patients using an experimental probiotic toothpaste, mouthrinse and toothbrush cleaner containing 5 × 107 cells of *B. subtilis, B. megaterium* and *B. pumulus* versus identical products lacking these microorganisms. Intra-group improvement of the gingivitis measurements was shown in both probiotic and placebo groups.

Acknowledgements and Conflict of Interest

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