The Effect of *Lactobacillus salivarius* SGL03 on Clinical and Microbiological Parameters in Periodontal Patients

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**Abstract**

The destruction of periodontal tissues during periodontitis is the result of the immune-inflammatory reactions to the bacteria of dental biofilm. Probiotics may reduce dysbiosis by the modification of the dental microbiome, which can influence the immune-inflammatory mechanisms. The aim of this study was to estimate the clinical and microbiological parameters, before and after 30 days of application of the dietary supplement containing *Lactobacillus salivarius* SGL03 or placebo. The study was conducted in 51 patients with stage I or II periodontitis during the maintenance phase of treatment. The clinical parameters and the number of colony forming units (CFU) of bacteria in supragingival plaque were assessed before and after 30 days of the oral once daily administration of the dietary supplement in the form of suspension containing *L. salivarius* SGL03 or placebo. There were no changes in the PI scores between and within the groups. The value of BOP decreased in both groups. In the study group the significant reduction of the mean pocket depth was revealed (from 2.5 to 2.42, \(p = 0.027\)) but without the difference between the groups. There were no significant changes in the number of bacteria within the groups. In the control, but not the study group, positive correlations were observed between the clinical parameters (variables) and the number of bacteria. The use of the dietary supplement containing *L. salivarius* SGL03 may reduce pocket depth despite the lack of changes in other clinical parameters and the number of bacteria in supragingival plaque.

**Key words:** probiotics, periodontal treatment, *Lactobacillus salivarius*

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**Introduction**

Gingivitis and periodontitis comprise a large group of diseases of a complex etiology (Hasan and Palmer 2014; Dahlen et al. 2019). Among them, plaque-induced gingivitis and periodontitis constitute diseases in which the primary etiological factors are bacteria in dental plaque and other types of biofilms present in the oral cavity (Bartold and Van Dyke 2019; Geisinger et al. 2019). Epidemiological studies show that these diseases pose a serious problem to public health, they may lead to systemic diseases such as diabetes and cardiovascular diseases (Caton et al. 2018; Dahlen et al. 2019). Therefore, prevention and treatment of periodontitis are crucial not only for the maintenance of teeth and oral health but also for the whole human.

Periodontal tissue destruction in the course of inflammation occurs due to both direct bacterial action and activation of indirect immune-inflammatory mechanisms in host tissues (Dahlen et al. 2019). The constant presence of pathogenic bacteria in the oral cavity, through a number of factors such as pro-inflammatory cytokines or proteolytic enzymes, supports mechanisms of chronic destruction of connective and bone tissue, which causes disease progression and hinders its treatment. For years research studies were mainly concentrated on the composition of dental biofilm microbiota and attempt to determine a specific bacterium eliminating which would allow effective treatment of the disease (Hasan and Palmer 2014; Dahlen et al. 2019; Proctor 2000). Initially, subgingival biofilm with a range of anaerobic bacteria was considered pathogenic, then...
species such as Aggregatibacter (previously Actinobacillus) actinomycetemcomitans or Porphyromonas gingivalis were identified as particularly pathogenic. In turn, studies by Socransky et al. (1998) proved the pathogenic role of not individual bacterial species, but rather bacterial complexes such as the red complex: P. gingivalis, Tannerella forsythia, and Treponema denticola. So far, however, no specific species have been identified that would be responsible for developing of the disease (Bartold and Van Dyke 2019). At present, nonspecific bacterial plaque is considered pathogenic in gingivitis, while in periodontitis, the role of additional risk factors, such as genetic and epigenetic factors, nicotinism, diabetes, as well as lifestyle and other environmental factors is emphasized (Hasan and Palmer 2014; Meyle and Chapple 2015; Bartold and Van Dyke 2019; Dahlen et al. 2019). The contemporary model of periodontal disease pathogenesis emphasizes the importance of dysbiosis and inflammation as the factors that directly lead to the destruction of periodontal tissues (Van Dyke et al. 2020). Simultaneously, concepts of so-called health-promoting biofilm appear, which are based on the theory of symbiosis between bacterial species and the specific response of the host to a given type of biofilm. According to these hypotheses, the development of periodontitis results from pathogenic bacteria, lack of beneficial bacteria, and host risk factors (e.g., genetic susceptibility (Haukioja 2010; Devine et al. 2015; Meyle and Chapple 2015; Dahlen et al. 2019)).

Bacterial biofilm reduction is still the basis of treating gingivitis and periodontitis. For many years, new therapeutic methods have been sought to increase biofilm reduction effectiveness, such as scaling and root planning (Geisinger et al. 2019). Supportive treatment includes local and systemic antibiotic therapy, local use of antiseptics, laser therapy, or photodynamic therapy. However, these methods are expensive, may cause undesirable general and local effects, and there is a lack of certain studies confirming their beneficial effects. Therefore, the use of probiotics (or pro- and prebiotics) seems to be particularly beneficial in this respect, which – by influencing the composition of the bacterial biofilm – may simultaneously contribute to the regulation of the immune-inflammatory response in the periodontium (Haukioja 2010; Gruner et al. 2016).

Probiotics are defined by World Health Organization (WHO) and Food and Agriculture Organization (FAO) as non-pathogenic live microorganisms, which – when administered inappropriately, e.g., as dietary supplements – improve the host’s health (Teughels et al. 2008; Zarco et al. 2012; Laleman and Teughels 2015). The term a biotherapeutic agent is used in the literature to describe microorganisms that accelerate the treatment or prevent complications of the disease, and their effectiveness has been scientifically proven, involving large groups of patients, randomized trials, placebo tests, and double-blind studies (Elmer et al. 1996; Mcfarland 2000). Attempts to use probiotics in dentistry date back to the 1990s and concern primarily prevention and treatment of caries and periodontal disease. The most popular probiotics belong to the genera Lactobacillus and Bifidobacterium (Gruner et al. 2016). However, the majority of studies in this area are based on in vitro experiments or small groups of subjects, while clinical trials involving large groups of patients are mainly retrospective. With the development of microbiological research, the effect of probiotics on oral microbiota as well as on clinical and immunological parameters in periodontal tissues is being studied increasingly.

The aim of the study was to assess the clinical (plaque index – PI; bleeding on probing – BOP; mean pocket depth – PD; maximal pocket depth – PD max) and microbiological parameters (colony-forming unit – FU, from supragingival plaque) in patients before and after 30 days of use of the dietary supplement containing Lactobacillus salivarius SGL03 in the form of an oral suspension, compared to the subjects receiving placebo.

**Experimental**

**Materials and Methods**

**Study group.** The study involved 51 patients (35 women and 16 men, mean age 54.3 years) treated at the Department of Periodontology and Oral Mucosa Diseases, Medical University of Warsaw. The study was conducted from July to December 2019 after obtaining the approval of the Bioethics Committee No. KB/79/2019. The characteristics of the study and control groups in terms of sex and age are presented in Tables I and II, respectively.

|   |   |   |
|---|---|---|
|   | Group A | Group B |
| F (females) | 19 (73.1%) | 16 (64.0%) |
| M (males) | 7 (26.9%) | 9 (36.0%) |
| Total | 26 (100%) | 25 (100%) |

Table I

|   |   |   |
|---|---|---|
|   | Group A | Group B |
| Age (years) | 55.35 | 53.28 |

Table II

SD – the standard deviation
The study comprised of patients diagnosed with periodontitis stage I and II (Tonetti 2018). The diagnosis was made based on clinical and radiological examination in accordance with the current classification of periodontal diseases (Caton et al. 2018). All patients were in the maintenance phase of periodontitis treatment and had completed the causal treatment phase at least three weeks earlier. No periodontal procedures were planned for any of the subjects during three months from the start of the study.

The following inclusion criteria were used: 1) age 25–65 years, 2) PD ≤ 5 mm; 3) interproximal clinical attachment level (CAL) ≤ 4 mm, 4) no lost teeth due to periodontal disease, 5) presence of minimum ten teeth and 6) minimum three weeks after scaling. Exclusion criteria were: 1) hypersensitivity to components of the preparation (lemon oil, rosemary oil), 2) nicotinism, 3) pregnancy or lactation, 4) antibiotic or other antibacterial therapy during the past 30 days, 5) the use of antibacterial rinses containing chlorhexidine for the last two weeks. The study was a randomized intervention study; a parallel-group assessment was carried out with the random selection of patients for the study and control group and researchers (double-blind trial). Patients were randomly divided into two groups A and B. The sample size for groups A and B was determined based on the expected values of PD max at the end of the study, i.e., 4.4 for group A and 5.0 for group B. The expected value of a standard deviation for both groups was set at 0.75. The confidence level was set at 95%, and the power of the t-test was set at 80%. Such assumptions gave the required sample size of 25 patients for each of the groups. After completing of the examination and statistical analysis of the results, decoding was performed, and group A was nominated as a study group (receiving preparation containing \textit{L. salivarius} SGL03 – Salistat SGL03), whereas group B as control (placebo). Products for both groups were identically factory-packed (prepared and delivered by the manufacturer). The composition of probiotic SGL03 and placebo are shown in Table III. It was recommended to use the product for 30 days, at least 30 minutes after evening toothbrushing. Study participants were instructed to prepare the suspension just before use, to hold it in the mouth for 30 seconds while spreading it over the surface of teeth and gums. After using the preparation, it was recommended to refrain from drinking water for 30 minutes, while from drinks other than water and eating meals – until the next morning.

\textbf{Clinical examination.} Each patient during the first visit (T0) and after 30 days of using \textit{L. salivarius} SGL03 (± 5 days) (T1) underwent a periodontal examination with the collection of material for microbiological testing. The periodontal examination was carried out by two trained for this study investigators using one type of 1 mm graduated probe (UNC probe 15 mm; Hu-Friedy, Chicago, USA) and it included the assessment of 1) dichotomous (yes/no) FMPI (full-mouth plaque index) according to O’Leary et al. (1972) on four tooth surfaces (i.e. distal, buccal, mesial, and lingual). The index was determined by dividing the number of surfaces with a plaque by the number of all tested surfaces; 2) dichotomous (yes/no) BOP index according to Ainamo and Bay (Ainamo and Bay 1975) on four tooth surfaces (i.e. distal, buccal, mesial, and lingual). The index was determined by dividing the number of surfaces with a plaque by the number of all tested surfaces.

\begin{table}[h]
\begin{center}
\begin{tabular}{|l|l|l|}
\hline
\textbf{Vial content} & \textbf{Medical product (Salistat SGL03)} & \textbf{Placebo} \\
\hline
Osmotic water & Osmotic water & \\
\textit{Glucoligosaccharides; prebiotic} & X & \\
Citric acid & Citric acid & \\
Potassium sorbate & Potassium sorbate & \\
Sodium lactate & Sodium lactate & \\
Vanilla flavor & Vanilla flavor & \\
Sucralose & Sucralose & \\
\textit{Lemone. o.} & X & \\
Rosemary e.o. & X & \\
\hline
\textbf{Vial cap} & Modified tapioca starch & Modified tapioca starch \\
Lactoferrin & X & \\
Live probiotic bacteria (\textit{L. salivarius} SGL03) & X & \\
Maltodextrin & Maltodextrin & \\
Magnesium salts of fatty acids & Magnesium salts of fatty acids & \\
Silicon dioxide & Silicon dioxide & \\
cholecalciferol/colecalciferol & X & \\
\hline
\end{tabular}
\end{center}
\caption{Composition of medical product and placebo (active ingredients are shown in bold).}
\end{table}

X – the lack of the ingredient in placebo
was assessed at six points of each tooth (i.e., distal-buccal, buccal, mesial-buccal, mesial-lingual, lingual, and distal-lingual). The index was determined by dividing the number of bleeding points by the number of all assessed points; 3) pocket depth (PD) was assessed at six points on each tooth as the distance in millimeters from the gingival margin to the bottom of the pocket. The mean value was calculated by dividing the sum of the measurements obtained by the number of measurement points. Two operators trained and calibrated until their results did not differ from standard. Only one examiner performed all (two) examinations of every patient.

The PI was evaluated on the day plaque was taken for microbiological testing. The supragingival plaque was collected, which is why patients were asked not to perform morning hygiene procedures. Patients were not advised to change their habits, and no hygienization or curative procedures were performed on them during the study.

**Microbiological examination.** For microbiological examination, a sample of the supragingival plaque was collected from each patient from contiguous surfaces of lower premolars (35/45) with a flat plastic instrument, approximately 1 mm³ in volume. All visits took place between 8.00 and 10.00 in the morning. Patients were advised not to perform hygienization procedures (including toothbrushing) prior to the visit. Each sample was placed in a sterile test tube containing 1 ml of thioglycolate buffer. The tubes were immediately transported to the laboratory, where they were mixed for 1 minute using a vortex mixer, with glass beads (5 beads/tube) to break down bacterial complexes. Each tube was then subjected to serial dilutions – from 1 : 1 to 1 : 1,000,000 – in phosphate-buffered saline (PBS). Successive dilutions, marked in an identifiable manner, were inoculated on a culture medium (Columbia agar with 5% sheep blood), and then incubated at 37°C for five days under anaerobic conditions (GenBag Anaer, bioMerieux). Thereafter, colonies were counted (CFU), and CFU/ml was determined. The microbiological examination was carried out at the Department of Dental Microbiology of the Medical University of Warsaw.

**Questionnaire.** Besides, after the study’s termination, patients completed an anonymous questionnaire regarding the taste of the preparation, ease of use, subjective assessment of the effect on the state of gingiva and mucosa, and adverse effects (no subject contacted researchers to report any adverse effects while using the preparation).

**Statistical analysis.** Statistical analysis was performed with Statistica v. 13 (TIBCO Software Inc., Palo Alto, USA). Data were presented as mean ± standard deviation (SD) and 95% confidence intervals. The student’s t-test was used for comparison of two independent groups for continuous variables. Relationships between clinical and microbiological parameters were assessed using the Spearman rank correlation coefficient (R). P values of less than 0.05 (p < 0.05) were regarded as statistically significant.

**Results**

All patients finished the study. The authors excluded one of them because of their doubts about whether he understood the instruction for use well. Finally, in group A there were 26 and in group B 25 patients. Demographic parameters (sex and age) of patients enrolled in study (A), and control (B) groups are shown in Tables I and II, respectively. In the study group, there were 19 females (73.1%) and seven males (26.9%), while in the placebo group – 16 females (64.0%) and nine males (36.0%). The study group’s mean age was 55.35 years, and in the placebo group – 53.28 years (p = 0.585).

The study (A) and control (B) groups did not differ at baseline in terms of plaque index, bleeding index, mean pocket depth, and maximum pocket depth (Table IV). The mean plaque index in the *L. salivarius* SGL03 group decreased from 55.38% to 51.61%, while in the placebo group – from 56.81% to 52.92%. The differences were not statistically significant (Table V).

| Table IV Initial mean values of clinical variables (parameters) (PI, BOP, PD max, mean PD) in *Lactobacillus salivarius* SGL03 (A) and placebo (B) group. |
|-----------------------------|-----------------------------|-----------------------------|-----------------------------|
|                            | Group A                     | Group B                     | p-value                    |
|-----------------------------|-----------------------------|-----------------------------|-----------------------------|
| Mean                        | Mean                        | Mean                        | 55.38                       | 20.50                       | 56.81                       | 16.14                       |
| SD                          | 20.50                       | 11.44                       | 20.30                       | 11.74                       |
| Mean PI                     | 55.38                       | 56.81                       | 0.783                       |
| BOP                         | 20.39                       | 20.30                       | 0.978                       |
| PD max                      | 4.88                        | 4.96                        | 0.825                       |
| Mean PD                     | 2.50                        | 2.46                        | 0.757                       |

SD – the standard deviation

in the placebo group – from 56.81% to 52.92%. The differences were not statistically significant (Table V). Similarly, the mean bleeding index decreased in both the *L. salivarius* SGL03 group (from 20.39% to 18.11%), and the placebo group (from 20.3% to 17.57%), but the differences were not statistically significant. However, the reduction of bleeding index in the whole group participating in the study (A + B) turned out to be statistically significant (from 20.34% to 17.84%, p = 0.011) (Table V). The average maximum pocket depth in the *L. salivarius* SGL03 group decreased from 4.88 to 4.58, while in the placebo group from 4.96 to 4.84. The differences were not statistically significant (Table V). In turn, the mean pocket depth in the *L. salivarius* SGL03 group underwent a statistically significant reduction.
from 2.50 to 2.42 (p = 0.027) (Table V). This parameter also decreased in the placebo group from 4.96 to 4.84, but the difference was not statistically significant. There was also no significant difference in this parameter between the \textit{L. salivarius} SGL03 and placebo groups (Table V).

The average number of bacterial colonies cultured from samples of supragingival plaque, collected from patients in the \textit{L. salivarius} SGL03 group, was $5.32 \times 10^7$ before the study and $8.77 \times 10^7$ after the study (Table VI). The difference was not statistically significant. In the placebo group, the average number of colonies was $1.18 \times 10^8$ before the study, and $1.09 \times 10^8$ after the study – the difference was also not statistically significant.

Correlations of clinical indices with microbiological parameters are shown in Table VII. In the study (A) group, a negative correlation was found between the maximum depth of periodontal pockets before and after treatment (PD max T0, PD max T1) and the number of bacteria before treatment (CFU T0) and the number of bacteria after treatment (CFU T1) in the study group (A). In the placebo (B) group, in turn, a positive correlation was observed between the following parameters: the pre- and post-treatment bleeding on probing (BOP T0, BOP T1) indices and the number of bacteria after treatment (CFU T1); the mean pocket depth both before and after treatment (Mean PD T0, Mean PD T1) and the number of bacteria after treatment (CFU T1); the mean pocket depth before treatment (Mean PD T0) and the change in the number of bacteria (ΔCFU); the maximum pocket depth before and after treatment (PD max T0, PD max T1) and the change in the number of bacteria (ΔCFU); the plaque index before treatment (PI T0) and the number of bacteria after treatment (CFU T1) as well as between the plaque index after treatment (PI T1) and the number of bacteria after treatment (CFU T1). Similarly, a positive correlation was found between the change in the plaque index (ΔPI) and the number of bacteria after treatment (CFU T1) and the change of the number of bacteria in the samples (ΔCFU).

Table VIII presents the survey results regarding the subjective evaluation of the taste of the preparation, convenience of use, effect on the state of gingiva and mucosa, and the adverse effects of the dietary supplement in the \textit{L. salivarius} SGL03 and placebo groups. Among the respondents, 61.5% rated the taste of \textit{L. salivarius} SGL03 as good, 26.9% as neutral, and for 11.5%, it was unpalatable. Similarly, 61.5% of patients thought that the preparation was convenient to use, 26.9% – neutral, and 11.5% – uncomfortable. Among patients using \textit{L. salivarius} SGL03 46.2% said that their gums had improved (vs. 60.0% placebo) and 53.8% that they had remained unchanged (vs. 40.0% placebo). No patient reported any deterioration of gingiva. Regarding oral mucosa condition assessment, 57.7% of patients reported that the condition had improved (vs. 52.0%...
placebo) and 42.3% (vs. 48.0% placebo) that it had not changed. No patient reported deterioration of mucosa. Three patients (11.5%) from the study group and one patient (4.0%) from the control group reported adverse reactions, and they were related to gastrointestinal disorders.

Discussion

The mechanism of probiotics in the oral cavity is not fully understood (Haukioja 2010; Umar et al. 2015; Laleman and Teughels 2015; Gruner et al. 2016; Seminario-Amez et al. 2017). In caries, probiotics are associated with reducing the number of colony-forming units (CFUs) of cariogenic bacteria (primarily Streptococcus mutans). Simultaneously, in periodontal disease, inhibition of periopathogens and so-called biofilm modification is observed (Iniesta et al. 2012; Montero et al. 2017; Barboza et al. 2020).

An interesting issue is the impact of probiotics on oral microbiota. Lactic acid probiotic bacteria produce antibacterial substances such as hydrogen peroxide, bacteriocins, and lactic acid, which provide a probiotic effect (Laleman et al. 2015; Takahashi 2015; Morales et al. 2016, Barzegari et al. 2020). In addition, by reducing levels of proinflammatory cytokines, elastase and prostaglandin E2 (PG E2), they inhibit an inflammatory response – humoral and cellular – in periodontal tissues (Haukioja 2010; Devine et al. 2015). It is also believed that probiotic bacteria compete with pathogens for adhesion surfaces and nutrients. L. salivarius, like other lactobacilli, is a species detected much more often in individuals with healthy periodontium compared to patients with periodontitis (Kõll-Klais et al. 2005). This species has strong antibacterial properties against pathogenic bacteria in the periodontium. The mechanism of its action is not entirely clear. Nissen et al. (2014) showed that L. salivarius inhibits the expression of toxins secreted by A. actinomyctencomitans, including leukotoxin A, thus inhibiting its virulence.

Research on the use of probiotics in periodontology can be divided into several groups. Laboratory
tests involve assessing probiotic bacteria’s effect on growth or functions (such as adhesion, coaggregation, secretion of antibacterial substances) of other bacterial strains in culture. Clinical studies concern assessing the effect of probiotics on clinical, microbiological, and immunological parameters in experimentally induced gingivitis or patients with disease – gingivitis or periodontitis. In these studies, probiotics are used as the only treatment or as an addition to conventional treatment (scaling and root planning). It is worth emphasizing that there is a lack of recommendations in the literature in which disease entities and phases of treatment probiotics should be used in periodontology. In this study, it was decided to use a probiotic in the maintenance phase of periodontal disease treatment to strengthen or maintain the effects achieved in the causal phase by modifying the microbiota’s composition and its impact on inflammatory, immunological and microbiological parameters in the periodontium.

According to literature, the most frequently evaluated clinical parameters comprise the plaque index (PI), bleeding on probing index (BOP), gingival index (GI), and pocket depth (PD), less frequently also the effect of probiotics on gingival crevicular fluid (GCF) volume. Many researchers – using various probiotic bacteria (e.g., Lactobacillus reuteri, L. salivarius, Streptococcus oralis, Streptococcus uberis, Streptococcus rattii) – reported an improvement in these clinical parameters used in periodontology; however, these differences often were not statistically significant in comparison to the control groups (Krasse et al. 2006; Shimauchi et al. 2008; Laleman et al. 2015).

In our study, no effect of probiotic on the plaque index (PI) was observed. In both groups, it oscillated around 50% before the test, and it slightly decreased after using the probiotic but remained within 50%. The differences between the initial and final visits and between the groups were not statistically significant. As the study group consisted of patients in the maintenance phase of treatment, it is not surprising, i.e., individuals with established hygiene habits. It was assumed that the probiotic administration is only intended to help maintain microbial balance within the bacterial biofilm, partially achieved after the causal phase of treatment, and to sustain this treatment’s effects. The relatively high average plaque index values (about 50%) probably resulted only from the lack of hygiene procedures on the day of the examination because they did not correspond to average bleeding indices. The bleeding index decreased in the study group (20.39% vs. 18.11%) and the control group (20.3% vs. 17.57%). Still, the differences were not significant in either of the groups. However, BOP reduction was significant in the whole group (study + control) of patients participating in the study (20.34 vs. 17.84). The lack of plaque index changes and the reduction of the bleeding index in the study and placebo groups indicated that it was not only the result of dental plaque.

It is worth mentioning that in literature, reductions in plaque index and bleeding index were found mainly in those studies where a probiotic was used as an adjunct to conventional therapy, i.e., during active treatment of gingivitis (natural or experimentally induced) and periodontitis (Vivekananda et al. 2010; Teughels et al. 2013; Morales et al. 2016). A statistically significant reduction in the mean pocket depth (PD) was observed in the study group in our study. However, there was no significant difference between the study and control groups. Penala et al. (2016) obtained similar results.

As mentioned in this paper, the study group consisted of patients in the maintenance phase of treatment. From the clinical point of view, reduction of pocket depth is an expected and beneficial therapeutic effect. For patients in the maintenance phase of periodontitis treatment, the most crucial goal is to maintain a low bleeding index, a symptom of active inflammation in the periodontium, and maintenance or progress of reduction of pocket depths obtained during the active treatment phase. In the causal phase of treatment, reduction of pocket depth mainly results from a reduction in the number of bacteria, thus reducing active inflammation in the periodontium. As a result, tissue hyperemia and swelling are reduced. In turn, further reduction of pocket depths in the maintenance phase of treatment may result from the regulation of additional destructive mechanisms in periodontal tissues. Maintaining favorable composition of bacterial biofilm, obtained from the elimination of bacteria in the causal phase, means that the inflammation does not recur, bleeding does not intensify, and inflammatory-immunological mechanisms are gradually modulated. In periodontium, healing processes begin to prevail over destruction processes.

As mentioned earlier, in our study, both plaque and bleeding indices were not significantly reduced, which could mean that the pocket reduction process included additional mechanisms. It may be indirectly confirmed by the results of a clinical parameter correlation analysis (Table VII). In the placebo group, positive correlations were observed between plaque and bleeding indices as well as the mean and maximum pocket depths vs. reduction in the number of bacteria; therefore, changes in the number of bacteria affected clinical parameters. Such correlations were not found in the study group, which may mean that the significant reduction in pocket depth observed in this group was not due to a change in clinical parameters and the number of bacteria but rather a change in biofilm composition and its effect on inflammatory and immunological parameters. In the study group, only a negative correlation
between the maximum depth of periodontal pockets and the number of bacteria before treatment was noted. It means that the greater the maximum depth of periodontal pockets before treatment, the smaller the number of bacteria were detected in tested samples. It may indicate that patients with the most advanced disease were very well motivated to maintain proper oral hygiene. This correlation disappeared after treatment, which may be associated with biofilm composition changes after using *L. alivarius* SGL03.

Microbiological testing of the oral cavity microbiota comprises several approaches, including qualitative or quantitative culture methods for detecting particular species (with the use of selective culture media) or groups of bacteria. PCR techniques are particularly useful as they enable detecting specific species of bacteria, including non-viable or non-cultivable microorganisms. Both probiotic and pathogenic periodontal species (e.g., *P. gingivalis* or *A. actinomycetemcomitans*) may be detected with this method. Metagenomic methods are increasingly used in dentistry, and they make it possible to detect the composition of microorganisms that make up the biofilm, as well as the percentage of individual types of bacteria (Xu and Gunsolley 2014; Dabdoub et al. 2016). These techniques also allow the discovery of new periopathogens, including bacteria that cannot be isolated using classical culture methods (Hiranmayi et al. 2017; Torres et al. 2019). A better understanding of oral microbiota composition and mutual interactions of microorganisms present in the course of the disease will allow for the use of more effective therapeutic procedures, including patients with periodontitis (Proctor et al. 2020).

Several types of samples are used in microbiological studies of the oral cavity; however, in periodontology, they mainly comprise specimens of supragingival and/or subgingival plaque.

According to the authors of the analysis concerning periodontal disease, the use of probiotics improves clinical parameters such as BOP, PD, GI, but not the number of colony-forming units (CFU) of bacterial periopathogens (Seminario-Amez et al. 2017). However, it should be remembered that the microbiology of periodontal pockets is very complex and comprises both periopathogens as well as aerobic, pioneering, and seemingly nonpathogenic bacteria.

A meta-analysis by Gruner et al. (2016), in which three papers on the effectiveness of therapy with probiotics containing Lactobacillus bacteria were evaluated, did not show their effect on the examined periopathogens: *A. actinomycetemcomitans*, *P. gingivalis*, and Prevotella intermedia. However, despite a reduction in gingival inflammation indices (GI and BOP), no impact of probiotics on the plaque index was observed in the analyzed studies. Therefore, the authors of the analysis conclude that its effect results from the influence on host response, not on bacteria themselves.

Several bacteriological studies revealed the effect of probiotic strains of Lactobacillus spp. on the reduction of *A. actinomycetemcomitans*, *P. gingivalis*, *P. intermedia*, and *T. forsythia* in the subgingival plaque of patients with periodontitis, with no significant effect on clinical indices in these patients (Mayanagi et al. 2009; Vivekananda et al. 2010; Iniesta et al. 2012; Montero et al. 2017). On the other hand, in the paper mentioned above by Hallström et al. (2013), the use of *L. reuteri* lozenges did not affect biofilm composition in experimental gingivitis. Similarly, in our research, no influence of the probiotic on the number of bacteria in supragingival plaque samples was observed, with a slight statistically not significant reduction in the number of bacteria in the placebo group and an increase (not statistically significant) in the number of bacteria in the study group. This rise may be related to different bacterial species composition in samples before and after applying the probiotic. Given the statistically significant reduction of pocket depths in the study group, it can be assumed that probiotic use could have a beneficial effect on changing the composition of dental plaque microbiota to microbiota with lower pathogenic potential. However, further studies are needed to evaluate the biofilm composition concerning specific bacterial species.

Long-term use of a probiotic may positively affect the composition of oral microbiota and interactions between individual types of bacteria and inhibit proinflammatory effects of periopathogens; however, it cannot replace daily hygiene procedures (Laleman et al. 2015). The authors of a systematic review of the literature regarding the effect of probiotics on experimentally induced gingivitis in humans concluded that probiotics may be an alternative to rinses containing chlorhexidine (CHX), which could have undesirable adverse effects (Barboza et al. 2020).

Although some studies do not show an improvement in clinical parameters (e.g., plaque and bleeding indices) after the use of probiotic, they indicate the possibility of its action by modulating inflammatory response, e.g., by reducing the level or activity of PGE2, proinflammatory cytokines or proteolytic enzymes (elastase, MMP-3 metalloproteinase) in gingival crevicular fluid (GCF) or saliva (Staab et al. 2009; Lee et al. 2015; Kuru et al. 2017).

Attention should be paid to the form of the supplement used in the study – Salistat SGL03 is a rinse solution, while the majority of supplements on the market are oral tablets or lozenges. This probiotic supplement (and the placebo) in the form of a solution enables the accurate distribution of the suspension on tooth and gum surfaces as well as the mucosa of the entire oral cavity. In addition, as recommended by
the manufacturer, bacteria constituting the contents of the package are kept in the mouth for about 30 seconds before being swallowed. It seems to be a much better and more effective form of probiotic application than tablets or lozenges. It also requires slightly increased patient involvement in the evening application procedure after thorough toothbrushing, which could have contributed to the improvement of clinical parameters in the placebo group.

The effects of probiotics reported in the literature on clinical parameters and inflammatory markers are variable, ranging from no effect to statistically significant decrease in PI, GI, and BOP indices and in GCF volume (Slawik et al. 2011; Iniesta et al. 2012; Hallström et al. 2013; Kuru et al. 2017). Interestingly, Kuru et al. (2017) reported that beneficial effects were also observed after cessation of toothbrushing for five days, which may indicate a beneficial effect of the probiotic in patients with temporarily reduced performance of hygiene procedures, e.g., for health reasons. Further studies are needed to clarify this issue.

It should be noted that in this study, only one probiotic preparation has been evaluated (containing L. salivarius SGL03), so our findings cannot rule out other effects of the use of other probiotics.

In this study, the authors also analyzed a questionnaire on subjective assessment of the probiotic preparation containing L. salivarius SGL03 (its taste perception, the convenience of use, the effect on the state of gums and mucosa as well as potential adverse effects of dietary supplement), in comparison to the preparation administered to the patients in the placebo group. Over 61% of patients in the study group were satisfied with both the taste of this dietary supplement as well as convenience of its use (in comparison to 80.0% and 76% in the placebo group, respectively). It is important to emphasize that the addition of probiotic strains of microorganisms may alter the taste and aroma of the final food product or dietary supplement due to the production of different metabolites (e.g., organic acids) during fermentation, and extended storage (Terpou et al. 2019). It may determine the patient's adherence to therapy, particularly during long-term treatment for several weeks or months. In this study, 46.2% and 57.7% of patients in the study group reported the improved effect on the gums and oral mucosa compared to 60.0% and 52.0% in the placebo group. As reported in the literature, probiotic dietary supplements increasingly used in dentistry – apart from their direct effects (e.g., inhibition of oral pathogenic microbiota) – may also contribute indirectly to the regulation of mucosal permeability and local immunity in the oral cavity as well as decreased gum bleeding and reduced gingivitis (Krasse et al. 2006; Anusha et al. 2015). There were adverse reactions reported in the questionnaire by three patients in the study group and one individual in the placebo group; however, it should be noted that no patient had reported these adverse effects during therapy or ceased the use of the preparation (probiotic or placebo) because of them.

Conclusions

The use of the probiotic-containing L. salivarius SGL03 in the form of an oral suspension in patients in the maintenance phase of periodontitis treatment could have contributed to a reduction in the periodontal depths pockets, with no change in other clinical parameters and the number of bacteria in supragingival plaque. The use of probiotics seems to be justified in the maintenance phase of treatment to sustain the microbiological balance obtained during its causal phase. They can then be an alternative to additional therapies with antiseptics. Further research is needed on individual bacterial species’ clinical and microbiological parameters to confirm the long-term effect of the preparation.

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Conflict of interest

The authors do not report any financial or personal connections with other persons or organizations, which might negatively affect the contents of this publication and/or claim authorship rights to this publication.

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