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Original Article

The effects of three different mouth rinses in a 4-day supragingival plaque regrowth study

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ABSTRACT

Objective: This study compares the effects of three different mouth rinses with respect to reducing *Streptococcus mutans* (*S. mutans*) colony counts on the teeth and tongue surfaces. **Materials and Methods:** In this study, comparison tests using the alcohol-free 0.1% chlorhexidine mouth rinse, alcohol-containing essential oil mouth rinse, and alcohol-free essential oil-containing mouth rinse were conducted. Patients were instructed to avoid mechanical cleaning with either a toothbrush or toothpick for 4 days. The first samples were collected from teeth surfaces and the dorsum of the tongue after a professional cleaning, and the second samples were collected after a 4-day plaque re-growth period. The supragingival plaque from the buccal surfaces of teeth #11, 14, 31, 34 as well as samples from the dorsum of the tongue, were assessed using the Dentocult® strips. **Results:** The Listerine® and Ondrohexidine® groups did not show any statistically significant differences between the values of the two samples ($P = 0.734$, $P = 0.307$). The MC® group and the control group showed significantly higher results than the first sample values. The effectiveness of the mouth rinses on *S. mutans* colony counts from the teeth surfaces were higher in the Listerine®, Ondrohexidine®, and Mouthwash Concentrate® groups. The difference between the first and second samples of the *S. mutans* colony counts from the tongue surface was found to be statistically significant, and *S. mutans* colony counts were higher than the first sample ($P = 0.015$). **Conclusion:** Alcohol and essential oil-containing Listerine® mouth rinse, alcohol-free Ondrohexidine®, alcohol-free essential oil-containing MC® mouth rinse had the same effect on *S. mutans* counts, higher than the 1% alcohol solution on teeth surface. They had the ability to maintain the *S. mutans* counts at the same level for 4 days in patients who did not perform any mechanical oral hygiene regimen.

Key words: Chlorhexidine, essential oil, mouth rinses, orthodontics, *Streptococcus mutans*

INTRODUCTION

Favorable skeletal and dental improvements are often achieved through orthodontic therapy with fixed appliances, although some undesirable side-effects to enamel may also result.[1] If oral hygiene is inadequate, areas of demineralization, called "white spot lesions," can appear.[2,3]

According to the acidogenic theory, the development of demineralization areas results from the increase in *Streptococcus mutans* (*S. mutans*), *Streptococcus sobrius, lactobacilli,* and *actinomyces* bacteria, which produce acid around the braces as they metabolize sugar.[4,5] Two to three weeks after plaque accumulation, white spot lesions appear on the buccogingival areas and may lead to patient dissatisfaction at the end of orthodontic treatment.[6,7] If these lesions progress to decay, cosmetic or extensive dental interventions are needed.

Some precautions can be taken to decrease the risk of demineralization and to strengthen the enamel structure.[8] A common strategy to improve mechanical plaque removal is to incorporate a chemotherapeutic agent, such as an antibacterial mouthrinse, into the oral hygiene regimen.[9]

Mouth rinses that contain essential oils effectively decrease the total number of microorganisms, such as *S. mutans*.[10-12] Considerable clinical trial evidence

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is available to show that oral hygiene is significantly improved when a mouth rinse containing essential oils, e.g., Listerine® (Johnson and Johnson, McNEIL-PPC, Inc., Skillman, NJ), which contains alcohol or another option as Mouthwash Concentrate® (One Drop Only GmbH, Stieffring, 14, 13627 Berlin – Germany) which is an alcohol free mouthrinse.

The clinical benefits of such mouth rinses are attributable to their bactericidal properties, which prevent or reduce supragingival plaque and gingivitis and decrease intrinsic malodor.[13] Chlorhexidine (CHX)-containing mouth rinses are accepted as the gold standard and are used as a positive control in most trials.[14,15] However, these rinses have side effects, such as enamel discoloration, mucosal erosion, taste disturbance, mouth burning, dry mouth, carcinogenic effects and the smoothing of composite materials, which limits their usage to approximately 5 weeks.[16] To prevent these side effects, alcohol-free CHX mouth rinses have been developed, one of them is Ondrohexidine® (One Drop Only GmbH, Stieffring, 14, 13627 Berlin-Germany), which is an alcohol free mouthrinse.

The purpose of this study was to compare the efficacies of three different mouthrinses in reducing *S. mutans* colony counts: Ondrohexidine®, Listerine®, and Mouthwash Concentrate®; the latter has not yet been compared to any other mouth rinses in a clinical trial.

MATERIALS AND METHODS

The study was conducted in 70 adults (46 females and 24 males) who were receiving orthodontic treatment.

Each volunteer was selected according to the following criteria: (i) good general health, (ii) no sign of destructive periodontal disease and no more than 4 mm gingival pocket depth, (iii) a minimum of 24 teeth, (iv) no antibiotic treatment during a 3-month period prior to the start of the trial, (v) no regular medication with anti-inflammatory compounds, (vi) no use of tobacco products, (vii) no pregnancy, (ix) no history of allergy to oral care products, (x) no regular use of oral antiseptics, (xi) no fluoride varnish application and (xii) no other dental treatment except orthodontic treatment. The patients had standard edgewise braces on their incisors, canines and premolars and standard edgewise bands on molar teeth. All subjects received written and verbal descriptions of the study design and signed informed consent forms. The study was approved by the regional Ethics Committee.

The subjects were randomly assigned to four test groups. On the odd numbered days of the month, the patients with the odd protocol numbers were assigned to the control group, and the even protocol numbers were assigned to Ondrohexidine® group. On the even numbered days of the month, the patients with odd protocol numbers were assigned to the Listerine® group, and the even protocol numbers were assigned to the Mouthwash Concentrate® group. The negative control group and the three test groups had braces for the last 2-6 months on both the lower and upper dental arches. The control group consisted of 10 patients while the other three test groups consisted of 20 patients each. The plaque on the teeth surface was colored using the plaque disclosing agent Eviplac® (Parana, Brazil), and patients with plaque indexes (PI) between 0 and 1.5 were selected [Figure 1]. PI was determined according to the criteria of the modified Quigley and Hein Plaque Index[17] (on a scale of 0-5). The patients received professional scaling and polishing to remove all the visible plaque, stains, and calculus. Following professional mechanical cleaning, pellicle samples were collected from the surface between the braces and gingiva from the upper right central incisor, upper right first premolar, lower left central incisor, and lower left first premolar teeth using a microbrush [Figure 2]. The samples from the four quadrants were then spread on square-headed plaque strips from the Dentocult® SM Strip mutans kit (Orion Diagnostica Oy, FI-02101 Espoo, Finland) [Figure 3a]. Then, subjects chewed paraffin gum to increase salivary flow, and saliva samples were collected from the surface of the tongue. These samples were spread on the round-headed Dentocult® Strip [Figure 3b], clipped with plaque strips and placed into tubes containing bacitracin [Figure 3c].

Prior to the trials, patients were informed of the design and limits of the study and instructed accordingly; these instructions included the type, amount, and usage frequency of the mouth rinse. They were also told not to perform any means of mechanical cleaning or to consume any chewing gum or similar products. This was a double-blind study, and the direction and distribution of experimental materials was performed by a secondary clinician. The tests were conducted based on a 4-day plaque accumulation period.[18] The first group of patients constituting the positive control group were directed to use 20 mL of essential oil-containing Listerine® mouth rinse twice a day for 30 s. Listerine® mouth rinse contains eucaliptol (0.092%), menthol (0.042%), methyl salicylate (0.060%), and thymol (0.064%) as

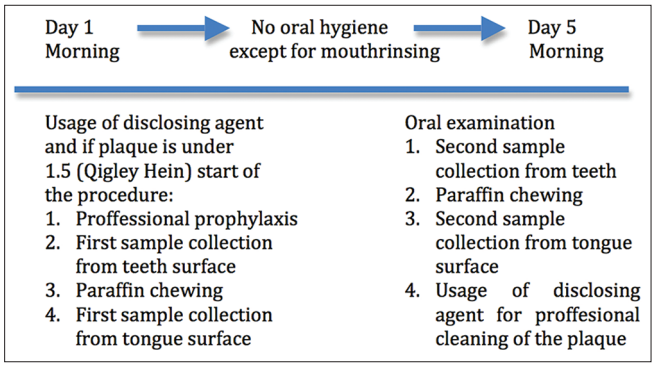

Figure 1: Outline of the clinical trial

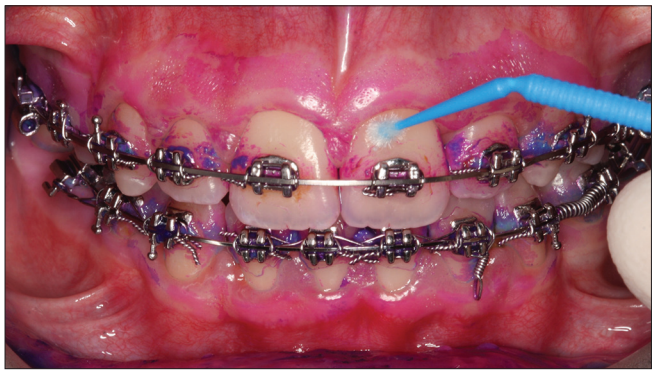

Figure 2: Method of plaque collection

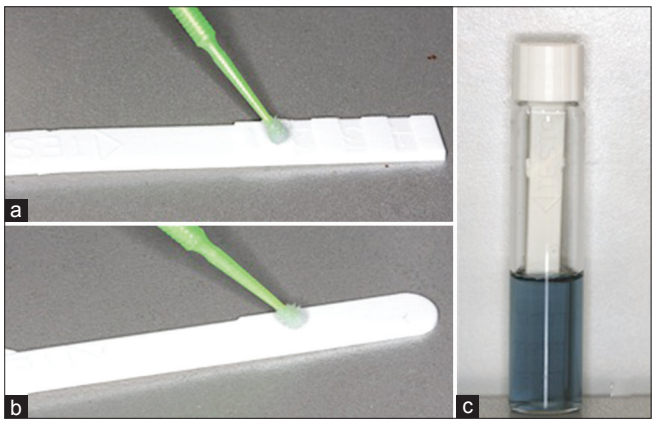

Figure 3: Plaque samples were collected using a microbrush (Microbrush International Ltd. Clogherane, Dungarvan Co., Waterford, Ireland) from the tooth surface (a) and tongue surface (b) and then spread on the site strip. The strips were attached to each other so that the sample-collection surfaces faced outwards, as well as to the cap of the culturing vial

active ingredients. Inactive ingredients include, water, alcohol (26.9%), benzoic acid, poloksamer 407, sodium benzoate, and caramel. The second group was directed to use 10 mL of 0.1% Ondrohexidine® mouth rinse twice a day for 30 s. The active ingredients of this alcohol-free mouth rinse are CHX digluconate (0.1%), potassium chloride (250 ppm), PEG-40 castor oil with hydrogen, and water with sorbitol and xylitol as flavoring. The third group was directed to use 30 mL of essential oil-containing Mouthwash Concentrate® 3 times a day for 30 s. The active ingredients of this alcohol-free mouth rinse are essential oil, water, menthol, thymol, eugenol, benzyl benzoate, and potassium hydroxide, with thyme and sage for flavor. The final group was designated as the negative control group and was directed to use 30 mL of 1% hydroalcohol solution 3 times a day for 30 s. The last rinse was performed in the evening of day 4. At the end of the test period, saliva, and plaque samples were collected in an identical fashion to the initial samples on the morning of the 5th day. Both sets of samples were analyzed for comparison. A total of 140 samples were tagged and kept in an incubator at 37°C for 96 h. According to the strip kit manufacturer, the incubation time should be 48 h; however, to avoid the lack of expression of *S. mutans* colonies, the manufacturer also advised to wait 96 h and re-evaluate the colony counts. Following incubation, *S. mutans* colony numbers were evaluated on a population density scale from 0 to 3 using the plaque and saliva templates included in a Dentocult® kit. The number of colony-forming units (CFU/mL) with characteristic morphology was screened and scored between 0 and 3. A score of 0 corresponded to zero CFU/mL (*S. mutans* below detection level); a score of 1 indicated 1-10 CFU and corresponded to approximately <10^4-10^5 CFU/mL; a score of 2 represented 10-100 CFU and corresponded to approximately to 10^5-10^6 CFU/mL; and a score of 3 represented more than 100 CFU and corresponded to more than 10^6 CFU/mL. The evaluation was blinded. One examiner gave the samples to the other without showing the sample names and recorded the scores. Each examiner repeated the scoring for inter- and intra-examiner scoring evaluation.

Statistical analysis

Inter- and intra-examiner reliability was evaluated by *kappa* (κ_w) analysis. The significance level was set at $P < 0.05$.

The test results from the 1st and 2nd sampling days were analyzed separately for each group. The analyses were conducted to compare the effects of 3 different mouth rinses with respect to their efficacy in reducing *S. mutans* colony numbers. Statistical calculations were performed with the NCSS 2007 Statistical Software (Number Cruncher Statistical System, Utah, USA) program for Windows. In addition to standard descriptive statistical calculations (mean, standard deviation, median and inter-quartile range), the Kruskal-Wallis and ANOVA tests were used for the group comparisons, and *post hoc* Dunn's multiple

comparison test was utilized for the two group comparisons. The Wilcoxon test was employed for the assessment of values from the 1st and 4th days. The Chi-square test was performed for the evaluation of qualitative data. The statistical significance level was set at $P < 0.05$.

RESULTS

Intra-examiner reliability in the scoring of *S. mutans* colony counts from the tooth surface (κ_w: 0.760, $P = 0.0001$) and tongue surface (κ_w: 0.790, $P = 0.0001$) was achieved, as well as inter-examiner reliability in the scoring of *S. mutans* colony counts from the tooth surface (κ_w: 0.615, $P = 0.0001$) and tongue surface (κ_w: 0.814, $P = 0.0001$). The patients who were randomly assigned to four groups were found to be balanced with respect to age ($P = 0.251$) and gender ($P = 0.699$) [Table 1].

Statistical analysis showed no significant difference in the group comparison of *S. mutans* colony count levels from the tooth surface in the first sample ($P = 0.700$). *S. mutans* colony count levels from teeth surfaces in the second sample showed a statistically significant difference ($P = 0.02$) between groups. The *S. mutans* colony count level of the second sample of the MC® group ($P = 0.015$) and control group ($P = 0.042$) were found to be significantly higher than those of the first sample, while the Ondrohexidine® and Listerine® groups did not present any significantly different results between the 2 time points ($P = 0.114$, $P = 0.307$) [Table 2].

The *S. mutans* colony count level of the control group was significantly higher than those of the Ondrohexidine®, Listerine® and MC® groups ($P = 0.015$, $P = 0.006$, $P = 0.048$, respectively) [Table 3]. There was no difference between the groups with regard to the *S. mutans* colony count levels from the tongue surface in the first sample ($P = 0.110$) [Table 4]. The comparison of the *S. mutans* colony count levels from the tongue surface of the second samples of the Ondrohexidine®, Listerine® and control groups did reveal statistically significant differences ($P = 0.017$). The two-group comparison revealed that the *S. mutans* colony count level of the control group was significantly higher than those of the MC®, Listerine® and Ondrohexidine® groups, whereas the comparisons between other groups did not reveal any statistically significant differences ($P > 0.05$), [Table 5].

DISCUSSION

During fixed orthodontic therapy, braces, bands, wires, and other attachments make it difficult for the patient to perform mechanical oral hygiene procedures; this difficulty results in plaque accumulation, which is the main cause of demineralization.[1] Following the placement of fixed appliances, plaque volume, and *S. mutans* colony counts were shown to increase; after the removal of the appliances, *S. mutans* counts decreased to normal values.[19,20] Soet *et al.*[21] concluded that the most notable etiologic factor contributing to decay formation was *S. mutans* because this bacterium

Table 1: Results (*P* values) of comparison of age and gender distribution of patients into 4 mouth rinse groups

Age (years)	MC® (15.30±2.34)		Ondrohexidine® (16.75±3.81)		Listerine® (17.15±3.37)		Control (15.80±2.30)		*P*=0.251
Gender									
Male	9	45.0%	6	30.0%	6	30.0%	3	30.0%	χ^2:1.42
Female	11	55.0%	14	70.0%	14	70.0%	7	70.0%	*P*=0.699

χ^2: Chi-square, Significance level $P<0.05$

Table 2: *Streptococcus mutans* colony count levels from the teeth surface on the first day after professional cleaning (first sample) and after 4th day (second sample): Results (*P* values) of Kruskal Wallis test * for comparison of mouth rinses

Teeth surface	MC®	Ondrohexidin®	Listerine®	Control	*P**
First sample					
Median (IQR)#	0.38 (0-1.25)	0 (0-1.25)	0.5 (0-1.44)	0.84 (0-2)	0.700
Mean±SD$	0.7±0.82	0.68±0.95	0.75±0.86	1±0.94	
Second sample					
Median (IQR)#	1.18 (0.65-2)	0.75 (0.25-1.5)	0.63 (0-1.75)	2 (1.44-2.31)	0.02*
Mean±SD$	1.38±0.87	0.94±0.8	0.88±0.85	1.8±0.87	
*P**	0.015*	0.114	0.307	0.042*	

*Significance level $P<0.05$, IQR#: Interquartile range, SD$: Standard deviation

produced more acid than *sobrius* and *mitis*. There is double risk with respect to caries formation for orthodontic patients; besides the problem of cleaning of the tooth surfaces, *S. mutans* also exhibit high adhesion to the surfaces of brackets.[22]

Antimicrobial mouth rinses are easy to use with fixed orthodontic appliances, and their ability to access most areas, including embrasures and around the appliances, makes them useful.[9,23] Based on current practices daily rinsing with a 0.05% sodium fluoride mouth rinse[24,25] is recommended for these patients. However patients' compliance with these directions is often unsatisfactory.[26] There are some undesirable side effects[16] related to the continuous use of antimicrobial mouth rinses; which is why patients are advised to use these mouth rinses on a short-term basis. Also, the clinicians are prompted to seek alternative mouth rinses that could be used for long spans of time. Essential oil (EO)-containing mouth rinses have been shown to produce a significant reduction in plaque endotoxin activity after long-term use.[27] In this context, the present study was designed to compare the effects of three different mouth rinses in the reduction of *S. mutans* colony numbers: Alcohol-free EO mouth rinse, alcohol-containing EO mouth rinse, and alcohol-free 0.1% CHX mouth rinse.

Table 3: *Post hoc* Dunn's multiple comparison tests to compare the differences between the teeth surface second sample values of the mouth rinses

Post hoc Dunn's multiple comparison test	Teeth surface/second sample
MC®/Ondrohexidine®	0.112
MC®/Listerine®	0.082
MC®/Control	0.048*
Ondrohexidine®/Listerine®	0.734
Ondrohexidine®/Control	0.015*
Listerine®/Control	0.006*

*Significance level $P<0.05$

Although some studies have stated that alcohol showed only a slight antibacterial efficacy against oral bacteria,[28-30] most of the mouth rinse preparations available contain alcohol in various amounts. While alcohol is used as a vehicle to dissolve and stabilize ingredients,[31] to prolong shelf life and as an antiseptic agent, some studies have reported that the presence of alcohol, especially, at high concentrations, results in increases in the effectiveness of mouth rinse solutions.[32,33] However, there are also other studies that report opposite conclusions.[33,34] A study by Van Strydonck *et al.*[35] concluded that a non-alcoholic mouth rinse containing CHX and 0.05% cetyl pyridinium chloride acted similarly to alcohol-containing 0.2% CHX mouth rinse with respect to their effects on plaque inhibition.

CHX mouth rinses are the gold standard for the inhibition of plaque formation;[14,15] however, most of these effective formulations have a high alcohol content,[16] and they are, consequently, inappropriate for some patients.[35-38] High amounts of alcohol and CHX are generally associated with side-effects.[16] As clinicians, we would prefer to recommend mouth rinses that do not have these side effects. Therefore, there is a need to identify alternative, alcohol-free solutions for sensitive patients and those who do not wish to use alcohol-containing mouth rinses, such as former alcoholics or those whose religions prevent the consumption of alcohol.

The alcohol-free EO solution in this study was tested against a negative control, as well as a positive CHX and a benchmark control, as in the study by Rosin *et al*.[39] Alcohol-containing EO mouth rinse was chosen as the benchmark control because the effectiveness of this EO-containing mouth rinse in controlling plaque and reducing the number of colonies of *S. mutans* has been demonstrated in many clinical trials.[18,40-46]

Table 4: *Streptococcus mutans* colony counts levels from the tongue surface on the 1st day after professional cleaning (first sample) and after 4th day (second sample): Results (*P* values) of Kruskal Wallis test * for comparison of mouth rinses

Tongue surface	MC®	Ondrohexidine®	Listerine®	Control	*P**
First sample					
Median (IQR#)	0 (0-1.75)	0 (0-2)	1 (0-2)	2 (0.75-2.25)	0.110
Mean±SD$	0.75±0,97	0.75±1.12	1.1±1.02	1.6±1.07	
Second sample					
Median (IQR#)	0 (0-1.75)	0 (0-1)	0.5 (0-1.25)	2 (1-2.25)	0.017*
Mean±SD$	0.8±1.06	0.5±0.83	0.78±0.94	1.7±0.95	
*P**	0.999	0.157	0.301	0.705	

*Significance level $P<0.05$, IQR#: Interquartile range, SD$: Standard deviation

Table 5. *Post hoc* Dunn's multiple comparison tests to compare the differences between the second tongue sample values of the mouth rinses

Post hoc Dunn's multiple comparison test	Tongue surface/second sample
MC®/Ondrohexidine®	0.389
MC®/Listerine®	0.924
MC®/Control	0.025*
Ondrohexidine®/Listerine®	0.307
Ondrohexidine®/Control	0.002*
Listerine®/Control	0.021*

*significance level $P<0.05$

In this study, the alcohol-containing EO mouth rinse was found to be effective while the alcohol-free EO rinse was not effective in inhibiting the *S. mutans* levels on tooth surfaces [Table 2]. The 2 EO mouth rinses contained different EO ingredients; therefore, it was not possible to discuss the effects of alcohol with regard to these two mouth rinses.

The alcohol-free solutions used in this study included a CHX solution and an EO solution. Although the CHX solution used in this study is alcohol-free, it still maintained the *S. mutans* CFU effectively [Tables 2 and 4].

In the 4 days of the non-brushing period, the *S. mutans* levels on tooth surfaces were maintained in groups in which alcohol-free CHX and alcohol-based EO-containing mouth rinses were used [Table 2]. The effect was similar for the two groups. The findings of this study, which involved alcohol-based EO mouth rinse and CHX, are consistent with those of other studies.[39,46] In a study by Riep *et al.*,[46] both 0.1% CHX-and alcohol-based EO, which were used as a 20 mL rinse for 30 s, were significantly superior to placebo and exhibited similar performances. Similarly, in the study by Rosin *et al.*,[39] a 0.12% Polyhexamethylene Biguanide (PHMB) mouth rinse was compared with a negative control placebo rinse (10% ethanol, flavor), a positive control 0.12% CHX rinse that did not contain alcohol, and a mouth rinse that contained EO and alcohol. Two similar 6-month controlled clinical studies also demonstrated that the EO mouth rinse and the CHX mouth rinse had comparable antiplaque and antigingivitis activities.[45,46]

In contrast, in a study by Moran *et al.*,[18] plaque scores with alcohol-based EO mouth rinses were significantly higher than with CHX; however, the dosage regimen of a 1 min rinse with 10 mL of the 0.2% CHX product as opposed to 30 s with 20 mL of alcohol-based EO mouth rinse might have been the reason for the high scores for the CHX mouth rinse in the direct comparison. Among the reasons for the inconsistency in the results and limitations of the studies are the uncontrolled mechanical cleaning procedures incorporated into the study methods, as well as the atypical prescriptions for the use of the mouth rinses.

There were some limitations of this study, which was the exclusion of patients who did not use the mouth rinses as notified in the prescriptions. Some other patients had no *S mutans* colonization because of high salivary buffer capacity, pH or flow rate.

The findings of this *in vitro* study emphasize as clinical relevance that patients undergoing fixed orthodontic therapy may use mouth rinse in addition to a mechanical oral hygiene regimen to reduce the colony counts of *S. mutans*. Long-term studies should be performed adjunct to mechanical oral hygiene procedures to ascertain the benefits of essential oil and alcohol free mouth rinses.

CONCLUSIONS

Essential oil-containing mouth rinses with and without alcohol, as well as CHX-containing mouth rinses, have the ability to maintain the *S. mutans* counts of the teeth at the same levels in patients who did not perform any mechanical oral hygiene regimen for 4 days.

The mouth rinses used in our study have the ability to maintain the *S. mutans* colony numbers at the same level on the tongue, even though no mechanical oral hygiene regimen is performed.

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