Original Article

Effects of xylitol impregnated toothbrushes on periodontal status and microbial flora in orthodontic patients

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ABSTRACT

Objectives: To investigate whether the use of xylitol-impregnated toothbrushes affects periodontal condition and microbial flora in orthodontic patients with poor oral hygiene.

Materials and Methods: Forty-four patients with baseline mean Turesky plaque index scores $\geq$1.5 were randomly divided into two groups. Half received xylitol-containing toothbrushes and the other half, xylitol-free toothbrushes. The periodontal measurements and saliva samples were taken at baseline (T0), 1 month later (T1), and 3 months after brushing (T2) to evaluate periodontal health and microflora changes. Periodontal status was assessed with plaque index (PI), gingival index (GI), and bleeding on probing (BOP) scores. Data were statistically analyzed with Mann-Whitney *U* and Friedman tests.

Results: All periodontal parameters significantly decreased from T0 to T1 and from T0 to T2 in both groups. The PI and GI scores reduced significantly in the control group, while BOP scores reduced in both groups between T1 and T2. Intergroup comparisons showed significant differences for BOP, PI, and GI at T0, T1, and T2 times, respectively. For microbial parameters, there were no statistically significant differences within groups from T0 to T1. Total bacterial counts significantly decreased in the xylitol group between T1 and T2. Decreases in *Streptococcus mutans* and total bacteria were significant in both groups from T0 to T2. No significant differences were found between the groups in microbial flora at any time.

Conclusions: A 3-month use of xylitol-containing toothbrushes showed almost the same changes and provided no positive effects on periodontal and microbial parameters compared to the control group. (*Angle Orthod.* 2020;90:837–843.)

KEY WORDS: Microbial flora; Periodontal health; Xylitol

INTRODUCTION

Orthodontic treatment aims to provide an esthetically pleasing smile with healthy surrounding tissues as well as ideal tooth alignment. Appliances used during treatment to achieve functional and esthetic goals negatively affect oral hygiene.[1] It has been shown that orthodontic attachments used in fixed orthodontic treatment create retentive areas that may cause plaque accumulation and make oral hygiene difficult.[2] Orthodontic appliances also lead to increases in the bacterial counts of *Streptococcus mutans* and *Lactobacillus* during treatment.[3]

Microbial dental plaque is considered the main etiological agent in the onset and progression of periodontal disease.[4] What first needs to be done to prevent inflammation is the mechanical removal of plaque. For this purpose, many researchers recommend the use of substances containing xylitol as a supplement to regular brushing procedures.[5–7]

Xylitol is a natural and five-carbon sugar alcohol found in low concentrations in various fruits and vegetables. This anti-caries agent is also widely found in many sugar-free products, especially in chewing gums, tablets, and lozenges. Stimulation of mineralization along with an increase in salivary flow are among the most common effects of xylitol and all other sweeteners. The most important feature of xylitol that is

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different from other sugar alcohols is that it cannot be fermented by oral bacteria. Additionally, it has been reported that xylitol reduces the amount of dental plaque by inhibiting the growth, metabolism, and polysaccharide production of *Streptococcus mutans*.[8,9]

In the literature, a limited number of studies have investigated the effect of xylitol in patients undergoing fixed orthodontic treatment using different forms of gums, tablets, and lozenges.[10–13] Additionally, a few studies have investigated the effects of xylitol-impregnated toothbrushes on plaque accumulation in young individuals.[14,15] However, no study has examined the effects of xylitol-impregnated toothbrushes on periodontal status and microflora during fixed orthodontic treatment. For this reason, the aim of this study was to evaluate the use of xylitol-impregnated toothbrushes in patients with poor oral hygiene receiving fixed orthodontic treatment in these parameters. The null hypothesis was that no differences would be observed between xylitol-containing and xylitol-free toothbrushes in their effects on periodontal and microbial parameters in orthodontic patients with poor oral hygiene.

MATERIALS AND METHODS

Trial Design

This study employed a parallel group design with two groups. Ethical approval was obtained from the Ethics Committee, Pamukkale University (16.12.2017/16). Informed consent was obtained from all participants or their parents.

Participants, Eligibility Criteria, and Settings

The study population was composed of 44 patients (22 males, 22 females) aged 12–18, with a mean age of 14.38 $\pm$ 1.96 years. Patients were included in this study according to the following criteria: (1) permanent dentition with no missing teeth or untreated decay; (2) no systemic or severe periodontal problem; (3) no previous orthodontic treatment; (4) brushing with right hand; (5) no antibiotic use in the last 3 months; (6) indication for fixed orthodontic treatment without extractions; (7) continued fixed multi-arch orthodontic treatment for at least 6 months; (8) presence of rectangular stainless steel archwires (0.017 $\times$ 0.025-inch); (9) same type of bracket system (metal, conventional) and ligation technique (wire ligatures); (10) scores of 1.5 and higher on the TureskyModified Quigley Hein Plaque Index.[16] After subjective plaque evaluation of patients, intraoral photographs were taken from the labial, occlusal, and palatal/lingual surfaces of all the teeth by means of a plaque disclosing agent (Mira-2-Ton, Hagerwing, Duisburg, Germany) for objective evaluation.

Interventions

Half of the patients received xylitol-containing toothbrushes (Happy Morning Xylitol, 605496, Hager Werken, Duisburg, Germany), and the others received xylitol-free toothbrushes (Happy Morning Xylitol, 605401, Hager Werken) and served as the control group (Figure 1). According to the manufacturer, nearly half of the total composition in the xylitol-free toothpaste was calcium phosphate and the other components were glycerin, sorbitol, sodium saccharin, carboxyl methyl cellulose sodium, menthol, pure water, paste, peppermint oil, lauric acid sodium, and silicon dioxide. The ingredients of toothpaste in the toothbrushes impregnated with xylitol consisted of the following components: sorbitol, sodium lauryl sulfate, sodium saccharin, alcohol, cellulose gum, aroma, xylitol, menthol and methylparaben. The details about the exact amount of xylitol in the toothpaste of these toothbrushes were not mentioned. All patients were warned not to use products containing any antimicrobial agents.

The patients were asked to use the disposable toothbrushes given to them for 12 weeks and to change their brushes every day. Patients were invited to the clinic every 15 days to receive new brushes. During the study, patients were asked to brush their teeth twice a day for 2 minutes according to the modified Bass method and were given standard oral hygiene training. No additional materials such as chains, coil springs, or figure-8 ligatures, that could have had a negative impact on oral hygiene, were applied during the study.

Outcomes

Clinical periodontal parameters and saliva samples were taken from all individuals at three different times. The measurements were performed before the toothbrush was given (T0), 4 weeks later (T1), and 3 months after the brushing protocol commenced (T2).

Periodontal Measurements

The periodontal measurements were performed at the upper and lower central incisors, lateral incisors, canines, and premolar teeth of all patients by the same investigator (SK). The gingival index (GI),[17] plaque index (PI),[18] and bleeding on probing (BOP)[19] scores were used for clinical periodontal parameters.

Amount of Microbial Colonization

The saliva samples were collected for each patient at the same times of day for the three different evaluation periods. Patients were informed to avoid brushing their teeth, drinking, or eating for at least 2

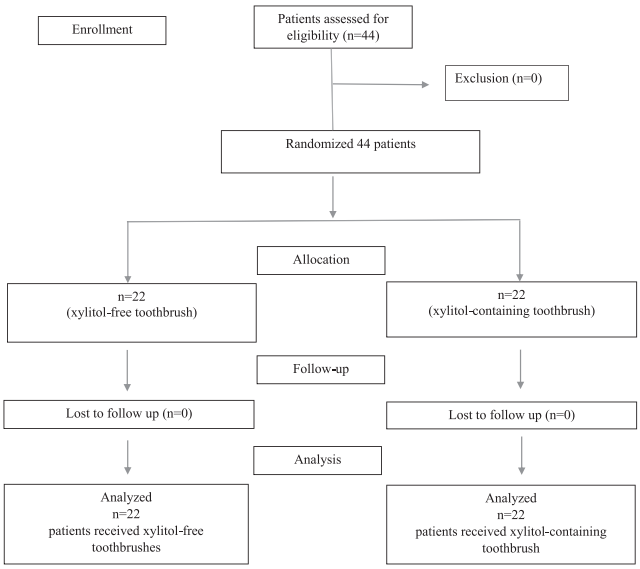

Figure 1. Consort flowchart.

hours before the collection of unstimulated 2-3 mL saliva samples. These samples were cultured and analyzed in the Department of Clinical Microbiology.

The inoculation was performed on Mitis-Salivarius agar (Difco Laboratories Inc, Detroit, MI, USA) containing 0.001% of Chapman Tellurite solution (Difco), 150 g sucrose, and 3.33 mg bacitracin (Sigma Diagnostics, St. Louis, MO, USA) per liter of agar for the number of *Streptococcus mutans*, and Rogosa agar (Merck, Darmstadt, Germany) for the number of *Lactobacillus*. The agar plates were incubated for 48 hours at 35 ± 2°C in a carbon dioxide incubator. The number of colonies was then determined under a stereomicroscope by the same researcher (İK). Total bacterial level was calculated by adding *Streptococcus mutans* numbers to *Lactobacillus* numbers. Results are expressed as colony-forming units per milliliter.

Sample Size Calculation

Power analysis was performed based on a 1:1 ratio between groups and showed that, for a power of 0.80 with strong effect size ($d = 0.80$) and at $\alpha = 0.05$ significance level, 42 patients would be required for the study. To overcome any missing data, the study sample size was adjusted to 44.

Randomization

Simple randomization was performed with coin flipping during the equal assignment of patients to study groups.

Blinding

The examiner who was blinded to group assignment carried out the microbial evaluation. Due to the study design that required use of disposable toothbrushes, blinding of the primary examiner was not possible. New brushes and oral hygiene instructions were given by the same researcher who performed the allocation of patients to the study groups.

Statistical Analysis

The records were statistically analyzed using SPSS version 24.0 (IBM Corp., Armonk, NY, USA). The Shapiro-Wilk test was used to test for normal distribution. Data were analyzed using Mann-Whitney *U* and Friedman tests for the comparison of parameters between groups and among times. All tests were performed with a significance level of $P < 0.05$.

RESULTS

Periodontal Measurements

Intragroup evaluation revealed that all periodontal parameters were significantly decreased in both the xylitol and control groups 1 month after brushing (Table 1, Figure 2). Between T1 and T2, there were no significant differences for PI and GI scores in the xylitol group, while the BOP values were significantly decreased; however, all parameters showed pronounced decreases in the control group in the same time interval. There were significant decreases in both groups for all parameters between T0 and T2 (Table 1).

Intergroup comparison showed that there were no significant differences between the initial periodontal measurements except for BOP scores. T1 and T2 periodontal measurements between the two groups demonstrated significant differences for PI and GI values, respectively (Table 1).

In the xylitol group, there were more pronounced decreases in BOP scores at the T0-T1 and T0-T2 time intervals. Plaque and gingival indices showed greater decreases in the control group at T1-T2. Also, greater decreases in gingival index scores were found in the control group for the T0-T2 time interval (Table 2).

Amount of Microbial Colonization

Microbial parameters for both groups at each timepoint are shown in Table 3. In the xylitol group, total bacterial counts significantly decreased from T1 to T2. There were statistically significant decreases for *Streptococcus mutans* and total bacteria counts in both the xylitol and control groups between T0 and T2. Intergroup comparison of all microbial parameters showed no significant differences at any timepoints. There were no significant changes in microbial flora parameters between the groups for any time intervals (Table 4).

Table 1. Comparison of Periodontal Parameters of Groups at Three Evaluation Times (T0, T1, T2) and Changes Between Times

		T0	T1	T2	
		Mean ± SD	Mean ± SD	Mean ± SD	*P*
PI	Xylitol	1.21 ± 0.49[a]	0.62 ± 0.23[b]	0.49 ± 0.37[b]	<.001
	Control	1.28 ± 0.52[a]	0.77 ± 0.27[b]	0.38 ± 0.12[c]	<.001
	P	.42	.03*	.40	
GI	Xylitol	1.15 ± 0.36[a]	0.80 ± 0.36[b]	0.81 ± 0.51[b]	.002*
	Control	1.30 ± 0.45[a]	0.93 ± 0.47[b]	0.49 ± 0.26[c]	<.001
	P	.27	.32	.01*	
BOP	Xylitol	74.77 ± 14.51[a]	42.05 ± 21.25[b]	23.64 ± 13.73[c]	<0.001
	Control	53.41 ± 17.00[a]	35.68 ± 17.41[b]	16.36 ± 12.36[c]	<0.001
	P	<.001	.28	.06	

SD indicates standard deviation; ns, non-significant; PI, plaque index; GI, gingival index; BOP, bleeding on probing.
[abc] Same letters show no significant differences within groups.
* Significant at $P < .05$.

DISCUSSION

Standard oral hygiene procedures are not satisfactory in most fixed orthodontic patients. Because fixed orthodontic treatment requires long-term plaque control, xylitol-containing products can be necessary to support mechanical plaque removal. For this purpose, many researchers have recommended the short-term usage of xylitol in the form of chewing gum, polyols, or lozenges in orthodontic patients.[10–12] Although the ability of xylitol to prevent plaque formation has been reported in previous toothbrush studies,[5,14] the current study evaluated the effects of xylitol-impregnated toothbrushes in orthodontic patients for the first time. More specifically, this study was performed to assess the potential of xylitol as a supplemental agent, exploring the inhibitory effects on bacterial colonization and plaque accumulation during rather low xylitol usage combined with regular brushing in patients undergoing fixed orthodontic treatment. Since patient attitudes toward oral hygiene maintenance, together with the ability to remove plaque, can change as a function of treatment duration, patients who had undergone at least 6 months of treatment were included to this study.

As the importance of brushing the teeth twice a day has been previously demonstrated,[20] xylitol-impregnated brushes were used in this manner and changed daily. In this way, the patients were exposed to approximately 0.02 g xylitol according to the manufacturers' information, which was below the recommended daily xylitol dose.[21]

For the primary periodontal variables of plaque and gingival inflammation, baseline measurements between the groups revealed no significant differences, which verified the random assignment of patients to the groups. On the other hand, comparison between BOP values showed a significant difference between the groups. Because of this difference, it was thought that clinical homogeneity could not be achieved. However, the differences may have been due to false positive readings caused by uncontrolled insertion pressures during recording in patients with poor oral hygiene.[22]

During this study, all periodontal parameters showed decreases as time progressed in both groups. It was obvious that there was a direct correlation between regular oral hygiene training and the decrease in the amount of plaque and gingival inflammation.[23] Additionally, T1 PI values of the xylitol group decreased significantly compared with those of the control group. In the xylitol group, patients were aware of the ingredients of their toothbrushes due to their informed consent. When determining the efficacy of toothbrushes, in short-term studies it has been observed that the clinical effectiveness of tested toothbrushes could be

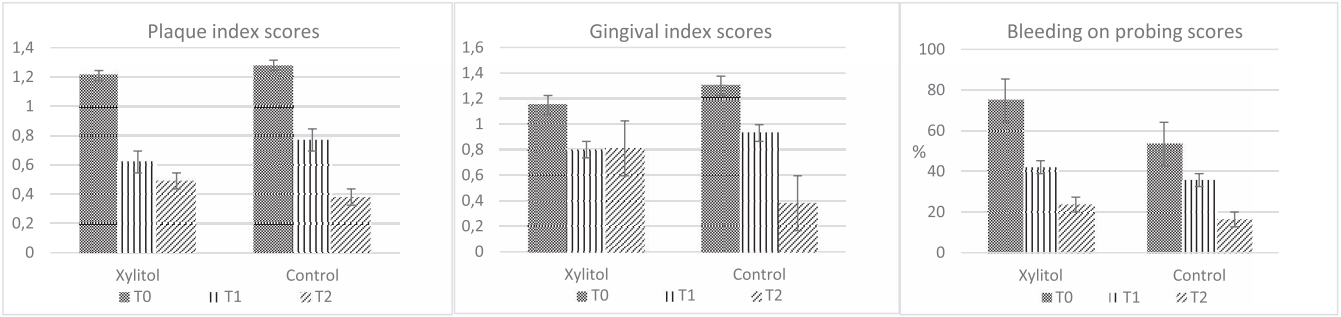

Figure 2. Periodontal parameters.

Table 2. Intergroup Comparisons of Changes for Plaque, Gingival and Bleeding on Probing Scores Between Different Time Intervals[a]

	Plaque Index Scores			Gingival Index Scores			Bleeding on Probing Scores		
	Xylitol	Control		Xylitol	Control		Xylitol	Control	
	Mean ± SD	Mean ± SD	*P*	Mean ± SD	Mean ± SD	*P*	Mean ± SD	Mean ± SD	*P*
T0-T1	0.59 ± 0.42	0.51 ± 0.47	.56	0.35 ± 0.25	0.37 ± 0.30	.80	32.73 ± 17.57	17.73 ± 22.61	.02*
T1-T2	0.13 ± 0.31	0.40 ± 0.24	.002*	0.00 ± 0.28	0.44 ± 0.31	<.001	18.41 ± 17.28	19.32 ± 16.5	.86
T0-T2	0.72 ± 0.49	0.91 ± 0.49	.19	0.35 ± 0.36	0.81 ± 0.36	<.001	51.14 ± 16.32	37.05 ± 22.87	.02*

[a] T0: Before the toothbrush was given; T1: 4 weeks after brushing; 4 weeks later; T2: 3 months after the brushing
SD indicates standard deviation.
* Significant at $P < .05$.

influenced by increased motivation levels of the participants, a phenomenon called the Hawthorne effect.[24] This seemed to have an impact during this study.

When the periodontal status of patients between T1 and T2 were evaluated, patients receiving xylitol-free toothbrushes demonstrated significant improvements in all parameters. However, plaque accumulation and gingival inflammation showed no pronounced changes in patients brushing with xylitol. An additional significant reduction for the control group was observed in this period that resulted in no positive effect of xylitol compared to the control group on periodontal parameters overall. For this time interval, the xylitol group showed significant differences in BOP values, perhaps due to the heterogeneous distribution of BOP scores at baseline between the groups.

From baseline to T2, GI, PI, and BOP decreased significantly in both groups. However, no significant differences were found between the groups. This can be explained by repeated oral hygiene procedures regardless of the content of the brushes used. Although the amount of plaque was expected to show greater decreases in the xylitol group due to inadequate colonization as a result of its inability to be metabolized by cariogenic bacteria,[25] xylitol did not provide superior effects on periodontal parameters. Consistent with these findings, Chi et al.[26] reported that xylitol was ineffective in patients who were at high caries risk despite xylitol's ability to decrease the volume of plaque by inhibiting the adhesivity of plaque. The lack of effect could be also explained by penetration of xylitol to plaque only by means of diffusion in patients with poor oral hygiene.[8]

Parallel to the changes in periodontal parameters, the numbers of *Streptococcus mutans*, *Lactobacillus*, and total bacteria decreased over time in both groups. However, no significant differences were observed between groups at any time interval. Contrary to these findings, Isotupa et al.[10] reported that the use of xylitol in the form of gum in orthodontic patients significantly decreased the counts of *Streptococcus mutans* after 4 weeks. In another study, Stecksen-Blicks et al.[12] demonstrated a slight but statistically significant decrease in salivary *Streptococcus mutans* counts in a xylitol group taking two tablets per day. These differences may be due to the high amount of xylitol in gum or tablets and higher daily doses. Due to the mechanical stimulation of saliva by chewing gum or taking a tablet, the findings of these studies may not be directly comparable to the current study.

In this study, xylitol-impregnated toothbrushes did not show a statistically significant difference in the reduction of *Streptococcus mutans* counts. Trahan et al.[27] reported that resistant *Streptococcus mutans* strains increased in saliva rather than in dental plaque. Later, it was suggested that non-xylitol-inhibited

Table 3. Comparison of Microbial Parameters of Groups at Three Evaluation Times (T0, T1, T2) and Changes Between Times

		T0	T1	T2	
		Mean ± SD	Mean ± SD	Mean ± SD	*P*
SM	Xylitol	2.05 ± 1.15[a]	1.70 ± 0.99[ab]	0.92 ± 1.05[b]	.01*
	Control	1.57 ± 0.88[a]	1.32 ± 0.93[ab]	0.83 ± 0.85[b]	.008*
	P	.13	.18	.75	
L	Xylitol	0.30 ± 0.30	0.18 ± 0.24	0.11 ± 0.12	.09
	Control	0.42 ± 0.66	0.39 ± 0.67	0.29 ± 0.45	.60
	P	.90	.50	.17	
TB	Xylitol	2.35 ± 1.24[a]	1.88 ± 0.96[a]	1.02 ± 1.02[b]	<.001
	Control	1.99 ± 1.24[a]	1.71 ± 1.19[ab]	1.12 ± 1.12[b]	.016*
	P	.35	.45	.89	

All mean and SD values should be multiplied by 10^5 to provide corrected values.
SD indicates standard deviation; ns, non-significant; SM, Streptococcus mutans; L, Lactobacillus; TB, total bacteria.
[a,b] Same letters show no significant differences within groups.
* Significant at $P < .05$.

Table 4. Intergroup Comparisons of the Changes For Microbial Parameters Between Different Time Intervals[a]

	Streptococcus mutans			*Lactobacillus*			Total Bacteria		
	Xylitol	Control		Xylitol	Control		Xylitol	Control	
	Mean ± SD	Mean ± SD	*P*	Mean ± SD	Mean ± SD	*P*	Mean ± SD	Mean ± SD	*P*
T0-T1	0.35 ± 1.04	0.25 ± 0.79	.79	0.12 ± 0.33	0.03 ± 0.73	.60	0.46 ± 1.13	0.28 ± 1.20	.61
T1-T2	0.78 ± 1.11	0.50 ± 1.19	.41	0.07 ± 0.25	0.10 ± 0.72	.48	0.86 ± 1.06	0.60 ± 1.58	.52
T0-T2	1.14 ± 1.55	0.74 ± 1.19	.35	0.19 ± 0.36	0.14 ± 0.41	.66	1.32 ± 1.55	0.88 ± 1.38	.32

All mean and SD values should be multiplied by 10^5 to provide corrected values.
[a] T0: Before the toothbrush was given; T1: 4 weeks after brushing; 4 weeks later; T2: 3 months after the brushing.
SD indicates standard deviation.

Streptococcus mutans was less virulent compared with strains inhibited by xylitol in long-term consumption.[28] Due to the lack of a key plaque-reducing mechanism of xylitol called the futile xylitol-5-phosphate cycle,[8] the decreases in bacterial counts of *Streptococcus mutans* and total bacteria were significant in both groups after 3 months of study. During this study, the numbers of *Lactobacillus* showed no pronounced differences at any time point in both groups as expected, because patients who had no active caries performed regular oral hygiene procedures. Although *Streptococcus mutans* is generally considered as among the most virulent of the cariogenic bacteria, several serotypes are found in oral microflora, and the total bacteria counts may be influenced by this situation and by changes due to the formation of mutans strains.

No previous study investigated the effects of xylitol-impregnated brushes on periodontal status and microbial flora during fixed orthodontic treatment. This study tested the effect of xylitol-impregnated toothbrushes on an orthodontic population with poor oral hygiene but the null hypothesis could not be rejected. In a 3-month period, toothbrushes containing xylitol had no different effect on periodontal status and microbial flora compared to xylitol-free toothbrushes in orthodontic patients with poor oral hygiene. These findings can be explained by the ingredients of the toothpaste, which had a low amount of xylitol, and the short exposure time during brushing, resulting in lower concentrations in saliva.

One of the limitations of this study included the lack of patient blinding. There is a need for new clinical studies with larger samples evaluating long-term use of xylitol-impregnated toothbrushes, taking into consideration the cost during fixed orthodontic treatment. It should be kept in mind that the effect of xylitol is dose-dependent, and knowing the actual amount contained in the toothpaste would have been useful to enable the reproducibility of studies regarding xylitol.

CONCLUSIONS

- From a clinical view, the effects of xylitol-impregnated and xylitol-free toothbrushes on periodontal status and microbial flora did not differ from each other in patients with poor oral hygiene undergoing fixed orthodontic treatment.
- Future studies using different dosages of xylitol or increasing the amount present in xylitol-impregnated toothbrushes should be performed.

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