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Original Article

Evaluation of the Efficacy of Different Cleaning Methods for Orthodontic Thermoplastic Retainers in terms of Bacterial Colonization

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ABSTRACT

Objective: This study investigated the antimicrobial efficacy of three different cleaning methods on *Streptococcus mutans* (SM) and *Lactobacillus* (LB) bacteria colonization *in vivo*. The three different cleaning methods were applied by volunteers on clear vacuum formed retainers (VFRs).

Methods: In this prospective, cross-over study, a total of 21 volunteers were included. All VFRs used by the volunteers were cleaned using three different cleaning methods in a sequence. These methods were peroxide-based cleanser tablets (PBCTs) plus brushing, control (only brushing), and vinegar plus brushing, respectively. The obtained salivary, VFR material, and periodontal data were statistically compared by factorial design repeated measures analysis of variance.

Results: The SM and LB bacteria counts on VFRs after using both PBCTs and vinegar were statistically similar ($p>0.05$), but bacteria counts were statistically lower than the control method ($p<0.01$). There were no statistically significant differences between the SM and LB bacteria counts in saliva samples taken before and after the application of the cleaning methods ($p>0.05$). Similarly, there were no significant differences between periodontal data obtained from plaque and bleeding indices at all study times. The periodontal pocket depth gradually decreased in the successively performed cleaning applications ($p<0.05$).

Conclusion: The application of PBCTs and vinegar to VFRs at sequential time intervals resulted in similar bacteria counts. The higher LB counts and similarly higher SM counts on the VFR samples indicate that mechanical cleaning only (control method) is not adequate to obtain hygiene. Salivary flora was not correlated with bacteria counts of VFRs.

Keywords: Bacterial colonization, *Streptococcus mutans, Lactobacillus,* thermoplastic retainers, retention

INTRODUCTION

Various retainers are used to maintain successful outcomes after an active orthodontic treatment. Vacuum formed retainers (VFRs) are frequently used due to their perfect esthetic features, small dimensions, ease of use and manufacture, and low cost (1, 2). However, these devices have disadvantages such as loosening over time, discoloration, fracture and crack formation, and limitation of the washing and buffering effects of saliva on teeth (2, 3). In addition, the presence of a thermoplastic retainer in the mouth affects the oral flora in favor of the cariogenic bacteria *Streptococcus mutans* (SM) and *Lactobacillus* (LB) (2). Therefore, when the pathologies related with microbial dental plaque are considered, the cleaning and hygiene of the VFRs are of great significance when it comes to oral and systemic health.

There are numerous mechanical and chemical cleaning methods used to remove the microorganisms accumulated on removable devices (4, 5). As the mechanical methods do not provide a sufficient antimicrobial effect on their own, they are recommended to be used together with chemical cleaning methods (5). Chemical products

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include household and commercially manufactured products (6, 7). Alkaline peroxide is a commercial disinfectant that provides mechanical cleaning by oxygen emission. In addition, alkaline peroxide-based tablets are having an antimicrobial effect similar to (8) or higher than (9) sodium hypochlorite, but causing less physical damage than sodium hypochlorite (9).

Among household products, white vinegar, shows antimicrobial and anti-tartar properties with its acidic features (10, 11). In addition, white vinegar is used in the cleaning of prosthodontic devices in dentistry due to its advantages, as being cheap and natural (11-13). There are many studies investigating the effects of fixed orthodontic treatment on oral microflora and periodontal health (14). However, there are a limited number of studies investigating the effects of cleaning methods on the devices used in the retaining treatment on oral flora and periodontal health after the active treatment is finished (15-17).

Table 1. Age and gender distribution of the individuals

		Age (year)		
Gender	**N**	**X± SD**	**min**	**max**
Female	16	16.63±3.14	13	25
Male	5	18.20±2.59	15	22
Total	21	17.42±2.87	13	25

N, number; X, mean; SD, standard deviation; min, minimum; max, maximum

This study comparatively assessed the effects of three different cleaning methods (peroxide-based cleanser tablets (PBCT) plus brushing, only brushing (control), vinegar plus brushing) under *in vivo* conditions for periodontal health parameter and SM and LB colony numbers, on the VFRs, and in saliva samples. In addition, the correlation between SM and LB counts in saliva and both VFRs was assessed. The null hypothesis was that there were no differences in the SM and LB colony numbers on the VFRs and in the saliva during the sequential application of PBCT plus brushing, only brushing (control), and vinegar plus brushing, in the same individuals.

This study was a prospective study with a cross-over design, approved by the ethics committee of the Republic of Turkey, Ministry of Health, Pharmaceuticals and Medical Devices Agency (21.04.2017.-71146310-511.06-E.89281). Written informed consent was obtained from all volunteers. The study was performed in the Department of Orthodontics, School of Dentistry, and in the Department of Microbiology, School of Medicine, Süleyman Demirel University, Isparta, Turkey, between May 2017 and December 2017. The inclusion criteria were the following: (1) undergoing the final stage of nonextraction orthodontic treatment with fixed appliances (the orthodontic attachments have not been removed yet), (2) having no active cavities, (3) being systemically healthy, (4) being a nonsmoker, (5) not having a carbohydrate-rich diet, (6) not undergoing dental fluoride treatment in the past 4 weeks, (7) not using a mouthwash containing anti-

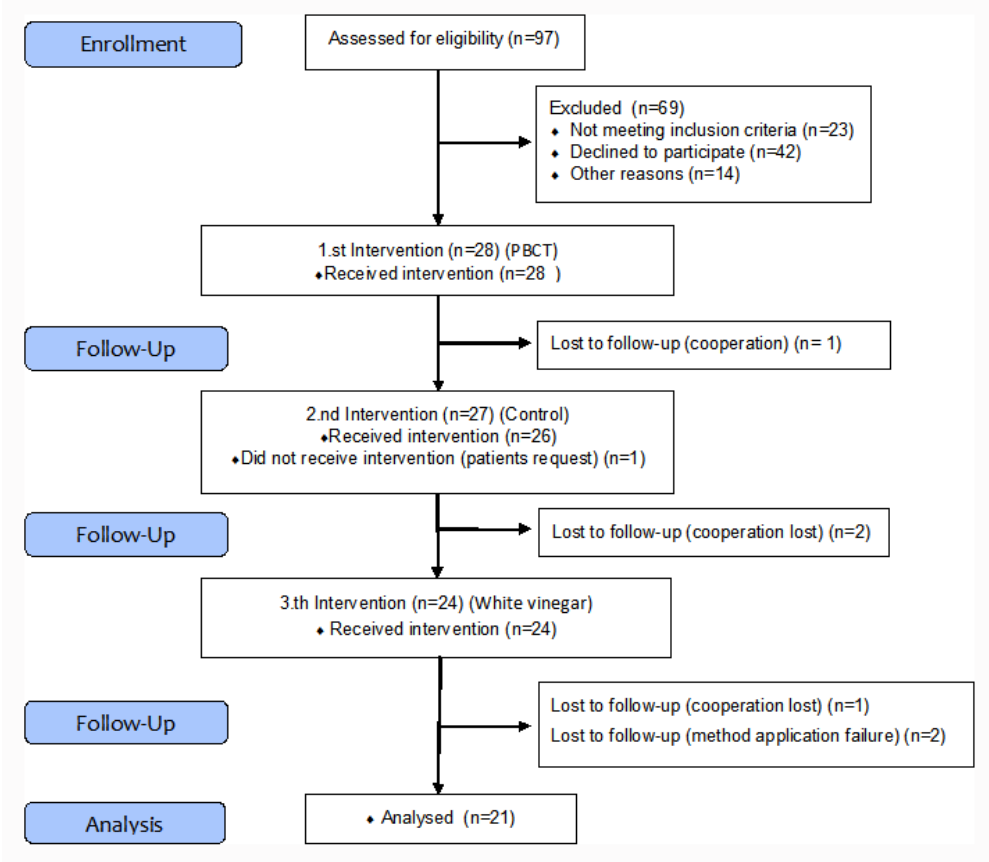

Figure 1. Flow diagram

Figure 2. Saliva samples at T0 were collected without VFRs, and at T1, they were collected while VFRs were in the mouth. Unstimulated saliva was obtained by sitting the patient upright, tilting the head forward, and draining the saliva to a sterile container for 10 minutes. The saliva was stored in a sterile container

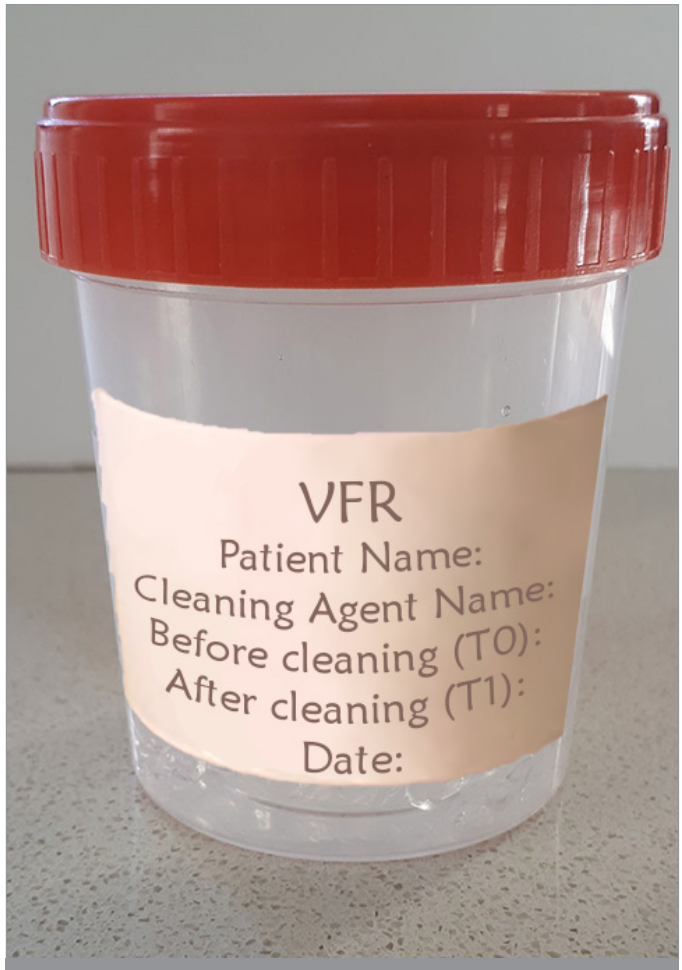

Figure 3. Storing the upper VFR in a sterile container with PBS. The name, surname, the period of experiment, cleaning agent name, and date were written

biotics or steroids in the past 2 weeks, (8) not being pregnant or lactating. In this study, a preliminary calculation was performed to obtain the sample size by using the G*Power software version 3.0.10 (Franz Faul Universität, Kiel, Germany). To achieve the 80% power, 20 patients were required to participate. Among the 95 individuals who satisfied the criteria of the study, 26 participated voluntarily in the study, but the data of 21 individuals were assessed (Table 1, Figure 1).

After all the orthodontic attachments were removed, dental scaling and polishing were performed, oral hygiene motivation was done, and the modified Bass technique was explained to the volunteers. Fixed lingual retainers were applied, and three pairs of upper-lower VFRs (DispoDent Sert Gece Plağı, Yağmur Dental, İstanbul, Turkey) were fabricated for each of them. In this study, VFRs were cleaned with three different cleaning methods: PBCT and brush, water and brush (without any cleaning solution-control), and vinegar and brush, respectively. Each cleaning method was applied to the new VFRs in a 4-week period, in succession and at sequential time intervals by each volunteer. But before the application of every new pair of VFRs and cleaning methods, one washout period (without using VFRs for 2 weeks) was applied. After the washout period, lower and upper VFRs were provided to the volunteers, and they were recommended to use them all day long, except for meals for 4 weeks. As the first cleaning method, the individuals were asked to use PBCT (Corega Tabs; GlaxoSmithKline, Brentford, Middlesex, United Kingdom) to their first VFR pairs, as explained in the prospectus. For each cleaning, a tablet was put into enough mild water to cover the upper and the lower VFR pair, and the VFRs were kept in this solution for 5 minutes. Then, the devices were brushed with a soft brush and rinsed with running water. After a 2-week washout period, the second cleaning method was applied. All surfaces of the second pairs of VFRs were brushed using only mild water and a soft brush, and then rinsed with running water (control method). After the third washout period, the third cleaning method was applied. 5% white vinegar (Ferfresh, Fersan, Izmir, Turkey) was put into a vessel to cover the third pairs of VFRs, and the devices were kept in this solution for 5 minutes. Later, all surfaces of the VFRs were brushed using a soft brush and rinsed with running water. For all cleaning agents, the applied procedures were repeated every day before bedtime. After the end of each cleaning method period (4 weeks), individuals cleaned their VFRs before bedtime, 1 day prior to each appointment, and arrived in the morning without having breakfast and brushing. At this appointment, the first unstimulated saliva samples were collected (Figure 2). Unstimulated saliva was collected by seating the

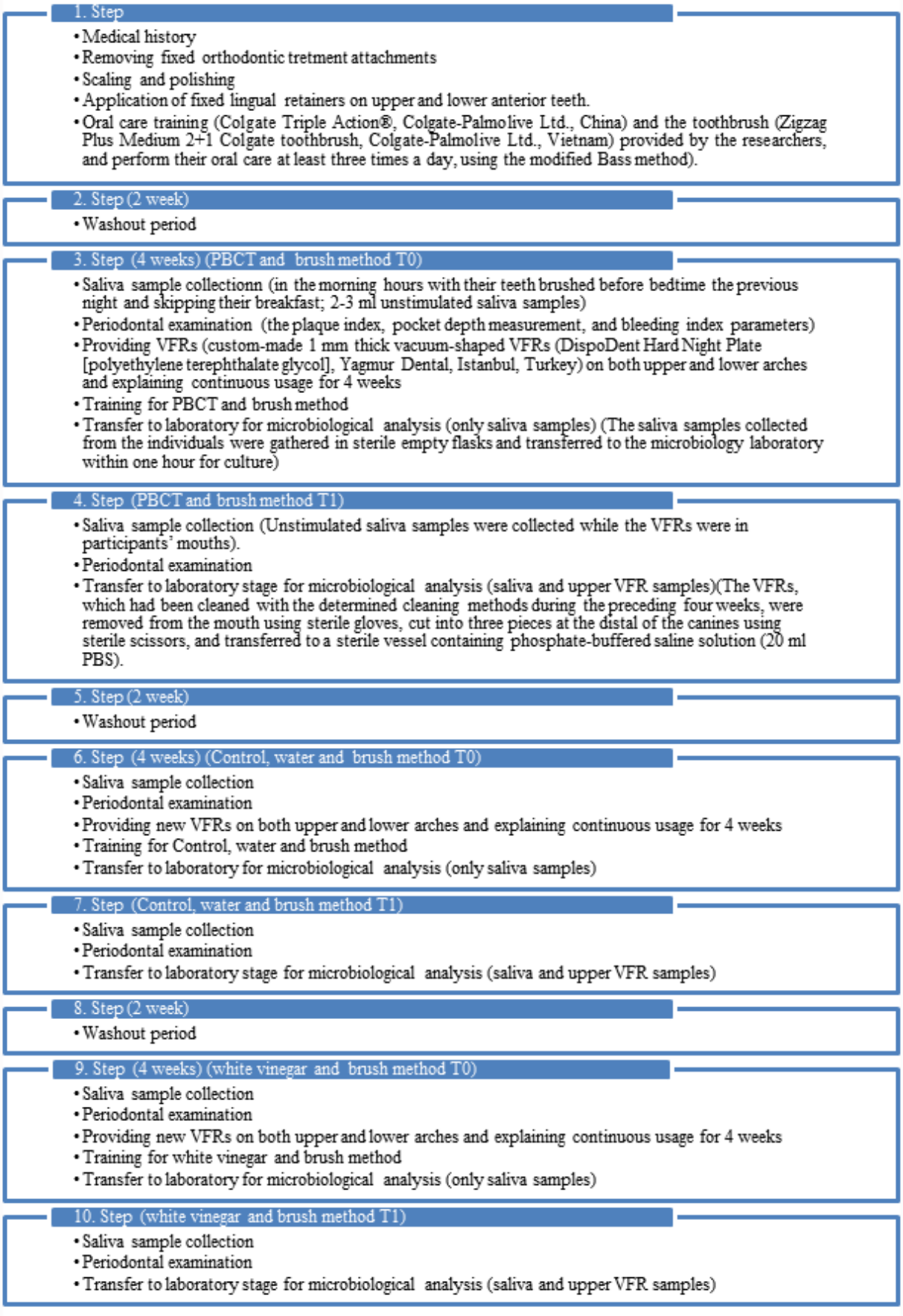

Figure 4. Clinical steps during the study period

Figure 5. Homogenization of the saliva sample in a vortex mixer and taking 1 ml of saliva sample

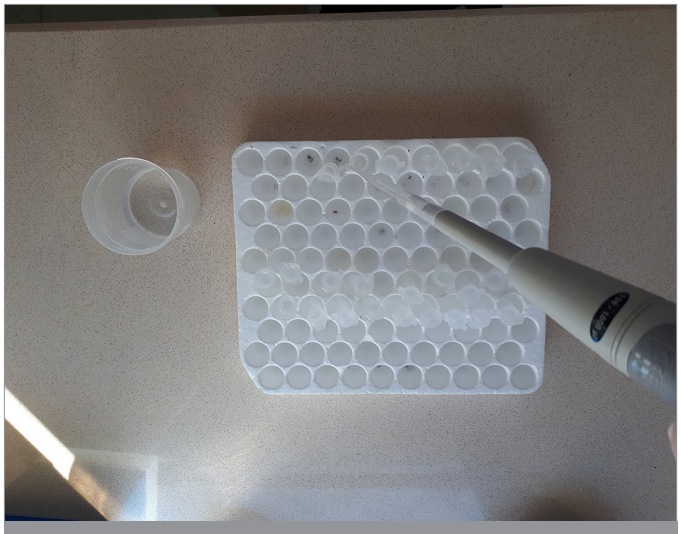

Figure 6. Dilution of the saliva sample

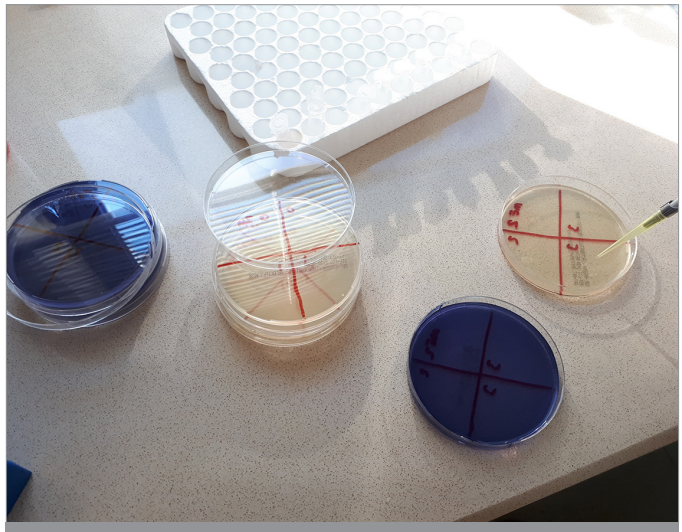

Figure 7. The Mitis Salivarius Bacitracin agar and Rogosa agar plates were divided into four equal parts with the acetate pen, and each dilution was numbered from 0 to 10. 10-µl diluted saliva specimens were cultivated to the plates numbered by dividing, according to the dilution degree. The same dilution was cultivated twice

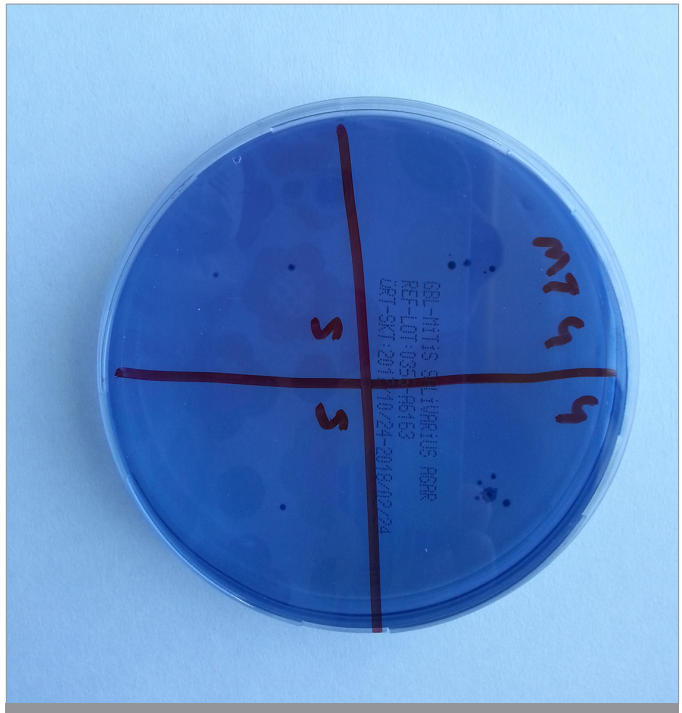

Figure 8. The plates were sorted according to their degree of dilution, and the colony counts were based on plaques where the colonies were fully counted with the naked eye

subject in an upright position at rest, tilting the head forward, and draining the saliva to a sterile container for 10 min. Then the upper and lower VFRs were removed, and upper VFR was divided into three pieces by using a sterile scissors. These were put into a sterile container with phosphate-buffered saline solution (PBS) (Figure 3). At each time, saliva samples from individuals were collected into a sterile empty container. Samples of the thermoplastic retainers were placed into a sterile container, containing PBS. Later, the plaque index, pocket depth measurement, and bleeding index (18) were obtained by one operator (FAA) as periodontal parameters. The study steps are presented in Figure 4.

Microbiological Analyses of Saliva and VFR Samples

The saliva samples that were transferred to sterilized containers were homogenized in a vortex mixer (Figure 5) (VELP Scientifica, Fisher ZX3 Vortex Mixer, Italy), and tenfold serial dilutions of 10^{-1} to 10^{-10} were prepared in a sterile 0.9% NaCl isotonic

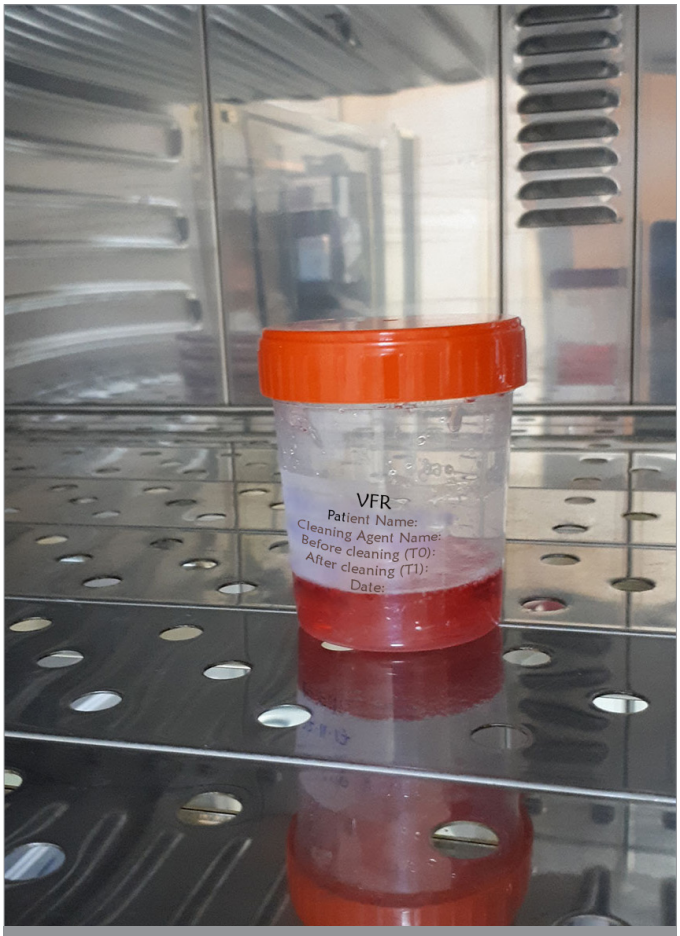

Figure 9. VFR samples were kept in closed containers, filled with 20 ml 0.25% Trypsin-EDTA in a 35°C±2°C incubator for 45 min

Figure 10. Homogenization of the VFR sample and Trypsin-EDTA in a vortex mixer and taking 1 ml of Trypsin-EDTA solution

solution, by means of taking 1 ml of saliva (Figure 6). Following the tenfold serial dilution, 10 µl saliva aliquots were plated in duplicate onto the mitis salivarius agar to obtain SM cultures, and the *Rogosa lactobacillus* selective agar for LB cultures (GBL, Istanbul, Turkey) (Figure 7). Samples were incubated in an anaerobic atmosphere (AnaeroPack-Anaero, Mitsubishi Gas Chemical Co. Inc., Japan) at 35°C±2°C for 48 h. The total number of colony-forming units (CFU) on each plate was counted after incubation (Figure 8).

The VFR samples were brought to the laboratory in a sterilized tube containing PBS, which was removed from the tube with a sterile syringe without touching the VFR samples, and 20 ml of 0.25% trypsin-EDTA solution was added to each tube. These VFRs were kept in this solution at 37°C for 45 min (Figure 9). Then, all specimens were homogenized in a vortex mixer (Figure 10). Following the homogenization, 1 ml of 0.25% trypsin-EDTA solution, including the VFR sample, was taken for the microbiological cultivation procedure. Next, microbiological cultivation was performed, as in the case of saliva samples.

SM and LB Colony Counts in Saliva and VFR Specimens

SM and LB colony counts were determined based on the dilution ratios of the plates on which the colonies were counted with the naked eye (Figure 8, 11). Each dilution was subjected

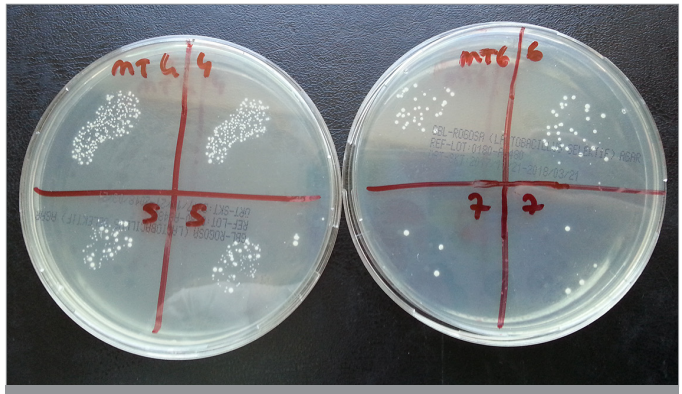

Figure 11. The plates were sorted according to their degree of dilution, and the colony counts were based on plaques where the colonies were fully counted with the naked eye

to duplicate inoculation of the plates. Therefore, the number of colonies on the plates of countable dilution was determined by taking the arithmetic average of the two cultures. The SM and LB colony counts belonging to saliva and VFR samples per individual were expressed as CFU in 1 ml (CFU/ml) of each sample. To determine the SM and LB colony numbers in a given 1 ml sample, the number of colonies determined on the plate was multiplied by the plate dilution factor and then divided by the volume transferred from the dilution tube to the culture plate.

CFU/mL = (colony count × dilution factor)/volume transferred from the dilution tube to the culture plate (ml)

Dilution factor = 1/dilution ratio (19)

The data originally measured in CFU were transformed to $\log_{10}$ for statistical analysis and reported as log CFU.

In this study, saliva samples and periodontal parameters were obtained after each washout period before the application of each cleaning solution (T0), 4 weeks after the application of each cleaning solution (T1). Upper VFR samples were obtained 4 weeks after the application of each cleaning solution (T1). Microbiological counts including SM and LB colony numbers were determined in saliva and on upper VFR samples.

Statistical Analysis

The obtained data were assessed by using Statistical Package for Social Sciences version 23.0 (IBM Corp., Armonk, NY, USA). To compare the duration of VFRs' wear, the one-way analysis of variance (ANOVA) test was used. In this study, periodontal parameters and saliva were evaluated by factorial design repeated measures ANOVA. The cleaning method factor had three levels; PBCT, control, and vinegar. In addition, the time factor had two levels: T0 and T1. The data obtained in terms of SM and LB colony numbers in saliva and on VFRs were analyzed by factorial design repeated measures ANOVA after log transformation. In the analyses, the cleaning method factor has three levels, as PBCT, control, and vinegar. The repeated measurements were conducted at the levels of the method factor. The Bonferroni multiple comparison tests were used in determining the differences between the factor levels at the end of the analysis of variance. Reverse-angle (arcsine) transformation was applied to the percentage results of the bleeding index, and analyzed by the factorial design repeated measures ANOVA. Arcsine transformation stabilizes variance and normalizes proportional data. The use of arcsine transformation, also known as inverse transformation or angular transformation, is useful in analysis of proportion data that tends to be skewed when the distribution is not normal. As the normal distribution was obtained for the plaque index data of the individuals, parametric tests were applied. The Pearson correlation test was used to determine the relation between the number of bacteria in the saliva and VFR samples. The significance level was assessed as 0.05.

RESULTS

The duration of the VFR wear by the patients during each cleaning method did not show any significant differences (p>0.05) (Table 2).

According to the factorial design repeated measures ANOVA, no interaction was found between cleaning methods and the time factor, and no differences were found in the cleaning method factor and in the time factor regarding the SM and LB bacteria counts (p>0.05) (Table 3). Similarly, no interaction was found between cleaning methods and the time factor, and no differences were found in the cleaning method factor and in the time factor (p>0.05) (Table 3). However, the differences in the cleaning methods for gingival pocket depth data were statistically significant (p<0.05) (Table 3). Both the SM bacteria counts on the VFR samples and the LB bacteria counts on the VFR samples were statistically significant with different cleaning methods (T1) (p<0.001). At T1, both SM and LB bacteria counts obtained for PBCT and vinegar cleaning methods were similar, and lower than the control method (p<0.05) (Table 4).

The correlation between the SM and LB bacteria counts in saliva samples at T1, and SM and LB bacteria counts on VFR samples at T1, was not statistically significant (p>0.05) (Table 5). A high correlation was found between the SM bacteria counts and LB bacteria counts on the VFRs at T1, for each cleaning method used in the study (Table 6).

Table 2. Duration of the VFR wear during each cleaning method

	Duration of VFR Wear (Hour)			
	N	x̄	SD	p
PBCT cleaning method	21	460.57	108.63	0.154
Control method	21	430.905	132.71	
Vinegar cleaning method	21	436.143	125.02	

x̄, mean; SD, standard deviation; N, number of volunteers; P, significance according to one-way ANOVA

Table 3. Descriptive statistics and the statistical evaluation of SM and LB counts in the saliva, total plaque index, periodontal pocket depth, and bleeding index according to applied cleaning methods

	PBCT				Control				Vinegar						
	T0		T1		T0		T1		T0		T1		P-Value		
	x̄	SD	x̄	SD	x̄	SD	x̄	SD	x̄	SD	x̄	SD	CMAT	CMA	T
Saliva SM Count	7.81	0.51	7.57	0.35	7.97	1.10	7.65	0.45	7.67	0.50	7.65	0.46	0.316	0.672	0.079
Saliva LB Count	7.70	0.49	7.49	0.33	7.88	1.19	7.52	0.45	7.50	0.49	7.50	0.45	0.605	0.605	0.054
Total Plaque Index	0.82	0.25	0.82	0.24	0.80	0.24	0.81	0.22	0.73	0.26	0.78	0.19	0.734	0.416	0.566
Periodontal Pocket Depth	2.06	0.33	2.06[a]	0.23	1.87	0.28	2.00[a,b]	0.25	1.89	0.31	1.89[b]	0.48	0.461	0.032	0.336
Bleeding Index	27.60	16.21	30.14	13.13	28.68	14.18	34.14	15.13	26.03	13.53	28.54	16.96	0.615	0.382	0.171

PBCT, peroxide-based cleanser tablets and brush method; Control, water and brush method; Vinegar, vinegar and brush method; T0, pre-application of cleaning method; T1, post-application of cleaning method; SM, *Streptococcus mutans*; LB, *Lactocbacillus*; x̄, mean; SD, standard deviation; CMAT, interaction between cleaning method application and time; CMA, cleaning method application factor; T, time factor; P, significance according to factorial design repeated measures ANOVA; superscript letters indicate the differences between the cleaning methods according to the Bonferroni multiple comparisons

Table 4. Descriptive statistics and the statistical evaluation of SM and LB counts on upper VFRs according to applied cleaning methods at T

	P							
	PBCT		Control		Vinegar			
	$\bar{x}$	SD	$\bar{x}$	SD	$\bar{x}$	SD	CMAT	CMA
VFR SM count	5.20[b]	0.63	5.99[a]	0.87	5.43[b]	0.82	0.455	0.000
VFR LB count	5.13[b]	0.74	5.90[a]	0.94	5.28[b]	0.86	0.149	0.000

VFR, vacuum formed retainer; PBCT, peroxide-based cleanser tablets and brush method; Control, water and brush method; Vinegar, vinegar and brush method, T1, post-application of cleaning method; SM, Streptococcus mutans; LB, Lactocbacillus; x̄, mean; SD, standard deviation; CMAT, interaction between cleaning method application and time factor; CMA, cleaning method application factor; T, time factor; P, significance according to factorial design repeated measures ANOVA, superscript letters indicate the differences between the cleaning methods according to the Bonferroni multiple comparisons

Table 5. Assessment of the correlation between the bacteria count in the saliva samples at T1 and the bacteria count on the VFR samples at T1

		PBCT VFR		Control VFR		Vinegar VFR	
		SM	LB	SM	LB	SM	LB
PBCT Saliva	SM	r=–0.111	r=–0.118				
	LB	r=–0.181	r=–0.151				
Control Saliva	SM			r=0.276	r=0.230		
	LB			r=0.329	r=0.311		
Vinegar Saliva	SM					r=0.125	r=0.259
	LB					r=0.053	r=0.192

PBCT, peroxide-based cleanser tablets and brush method; Control, water and brush method; Vinegar, vinegar and brush method; VFR, vacuum formed retainer; T1, post-application of cleaning method; SM, *Streptococcus mutans*; LB, *Lactocbacillus*; the data related to the SM and LM counts on the VFR samples were exposed to logarithmic transformation; r, Pearson's correlation coefficient; 0.7≤r high correlation; 0.3<r<0.7 moderate correlation; 0.3≥low correlation

Table 6. Assessment of the correlation between the SM and LB bacteria counts on the VFR samples at T1

		LB Count on VFR T1		
Bacteria Counts		PBCT	Control	Vinegar
SM count on VFR T1	PBCT	r=0.910**		
	Control		r=0.988**	
	Vinegar			r=0.921**

PBCT, peroxide-based cleanser tablets and brush method; Control, water and brush method; Vinegar, vinegar and brush method; VFR, vacuum formed retainer; T1, post-application of cleaning method; SM, *Streptococcus mutans*; LB, *Lactobacillus*; the data related to the SM and LM counts on the VFR samples were exposed to logarithmic transformation; r, Pearson's correlation coefficient; 0.7≤r high correlation; 0.3<r<0.7 moderate correlation; 0.3≥low correlation; **; p<0.01

DISCUSSION

There are various appliances for maintaining the achieved ideal dental and skeletal outcomes, as a result of an active orthodontic treatment. VFR, one of the widely utilized removable appliances used in retention, is routinely applied in orthodontic clinics since it is easy to prepare, cheap, and esthetically preferred (1).

In previous studies, the accumulation of microorganisms such as SM, *Streptococcus sobrinus*, LB, *Staphylococcus epidermidis* and *Staphylococcus aureus, Enterobacteriaceae,* and *Candida* on removable orthodontic devices was investigated (20-22). Among these, the most cariogenic microorganisms are SM and LB. Therefore, the effect of the three different cleaning methods on the SM and LB bacteria counts were evaluated in our study. Periodontal parameters were also assessed, because the SM and LB counts may be affected by the oral hygiene status of the individuals and the presence of oral devices.

The duration of the VFR wear during the application of each cleaning method in our study was similar. It has been reported that the number of the microorganisms increased when the duration of the removable device usage increased (23). The similarity in the duration of removable device usage ensured that the conditions in the application of the cleaning methods were similar and that the effect of cleaning agents could be comparable (22).

Although, the periodonto-pathogenic bacteria counts after the orthodontic treatment were frequently investigated (17, 24-26), a limited number of studies were about the bacteria counts in the saliva (15, 16). Kim et al. (17) and Sallum et al. (24) stated that there was a significant decrease in some periodonto-pathogens and the total number of bacteria after the removal of orthodontic appliances. On the other hand, Jung et al. (16) reported that, while the total number of bacteria significantly decreased in the saliva samples taken 5 weeks after the removal, the numbers of SM and *Streptococcus sobrinus* increased due to the usage of removable retainers. In our study, the saliva samples had been collected 2, 6, 8, 12, 14, and 18 weeks after the removal of fixed orthodontic attachments, and no statistically significant differences were found in the SM or LB bacteria counts in the saliva before or after the application of cleaning methods. Similar bacteria counts in the saliva during our study period may be a result of the performed dental scaling and polishing application. In addition, the oral hygiene motivation of volunteers could have been increased by the given oral hygiene training after ending their fixed treatment. The participants who were aware that they were monitored during the study might have exhibited increased motivation (the Hawthorne effect). Furthermore, during the 2-week washout period, the oral flora might have got over the effects of the fixed treatment and attained their normal composition.

The similar plaque index scores assessed at all study periods for all three cleaning methods show that the oral hygiene status for all individuals was similar during the whole study. Similar to our findings, Kim et al. (17) obtained similar plaque index scores for the 1st, 5th, and 13th weeks after the removal of orthodontic attachments. In contrast, Yáñez-Vico et al. (27) found lower plaque index scores 15 days after the removal session than in the control group who had never received the orthodontic treatment. These conflicting results could be caused by the patients who improve their oral hygiene before the appointments.

In this study, the difference between the pocket depth data for cleaning methods was statistically significant ($p<0.05$). The cleaning methods applied were PBCT, control, and vinegar, and the periodontal pocket depths were found to decrease following this order, with a statistically significant difference between them ($p<0.05$). Although a decrease in the pocket depth with time seems to be related with the change of the cleaning agent, the real reason might be the decrease of gingival hyperplasia caused by the orthodontic treatment. The removal of fixed treatment devices enables oral hygiene to be performed more easily. The studies reported that the increased pocket depth did not change significantly 4 weeks after the removal sessions (28) and that it either decreases (29, 30) or recovers (30) in 2 years.

The bleeding on probing index scores was similar at all study times for the three cleaning methods. In the literature, it was reported that the bleeding indices decreased after the removal session (28). These conflicts might have resulted from the increased oral hygiene motivation of volunteers during our study and the performed washout period before the experiment.

When the SM bacteria counts on the VFR samples are considered, it is observed that after the application of different cleaning methods, the difference between the SM bacteria counts on the VFR samples were statistically significant ($p<0.001$). While the SM bacteria count on the VFR samples cleaned with PBCT and vinegar was statistically similar ($p>0.05$), the SM bacteria count on the VFR samples cleaned with the control method was statistically higher than in other methods ($p<0.05$). When VFR samples were cleaned with different cleaning methods, the difference between the LB counts was statistically significant ($p<0.001$). The LB count on the VFR samples cleaned with PBCT and vinegar is statistically similar, while the LB counts on the VFR samples cleaned with the control method are statistically higher than other methods ($p<0.05$). The higher SM counts and similarly higher LB counts on the VFR with the control method might indicate that only mechanical cleaning (control method) is not adequate to obtain hygiene. This result is compatible with literature (5). On the other hand, contradictorily to our PSB cleaning agent and vinegar result, it was stated that vinegar has a less expressed antimicrobial effect than the PSB cleaning agents (7). Contrary to that finding, there are also studies reporting that vinegar is more effective (11, 13). These contradictory results could be caused by the fact that different kinds of microorganisms were investigated or that different chemical agent brands were compared. Different application procedures and research designs used in the studies could be the other reasons for this conflict.

Although it has been reported in the literature that the disinfection of the removable appliance decreases the number of microorganisms in the saliva (31), we did not determine any correlations between the number of bacteria on the VFR sample and saliva samples. The reasons for these contradictory results might include comparing different cleaning solutions and investigating different VFR materials.

A high correlation was found between the SM counts and LB counts separately for the PBCT, control, and vinegar cleaning methods (PBCT, $r=0.910$; control, $r=0.988$; vinegar, $r=0.921$). This is because the VFR was fabricated with vacuum and had many indentations, and these surfaces form accumulation areas for microorganisms. In addition, the increase in the roughness of the VFR material from intraoral use increases dental plaque accumulation. The increase of SM on a surface decreases the pH of the environment. The decrease in the oral environment's pH causes an increase in the number of LB. The coexistence of these bacteria in the oral environment causes the number of the bacteria to increase. However, to the best of our knowledge, there are no studies in the literature investigating the correlation between the number of the SM and LB bacteria accumulating on the removable orthodontic devices.

Investigating a limited number of cleaning agents and bacterial species was the limitation of this study. In future studies, other commercial and natural cleaning agents can be compared with regard to their microbiological effects. In addition, surface property changes of biomaterials with the use of these cleaning agents can be investigated in a long term.

CONCLUSION

Both the SM and LB counts were similar on the VFRs cleaned with PBCT and vinegar, but bacteria counts were statistically lower than in the control method. The higher LB counts and similarly higher SM counts on the VFR samples indicate that mechanical cleaning only (control method) is not adequate to obtain hygiene. The SM and LB bacteria counts in the saliva samples at the T0 and T1 were similar, independently from the cleaning method used. A statistically significant decrease was recorded for the pocket depth scores during the study.

Ethics Committee Approval: Ethics committee approval was received for this study from the Ethics Committee of the Republic of Turkey, Ministry of Health, Pharmaceuticals and Medical Devices Agency (21.04.2017.-71146310-511.06-E.89281).

Informed Consent: Written informed consent was obtained from the patients who participated in this study.

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