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Original Article

Contamination of Low Frictional Elastomeric Ligatures by *Streptococcus mutans*: A Prospective RT-PCR and AFM Study

Caglar Dagdeviren[1], Aysegul Gulec[1], Fahriye Eksi[2], Mustafa Saglam[2], Mehmet Kahraman[3]

[1]Department of Orthodontics, Dentistry Faculty, Gaziantep University, Gaziantep, Turkey
[2]Department of Microbiology, Medical Faculty, Gaziantep University, Gaziantep, Turkey
[3]Department of Chemistry, Art and Science Faculty, Gaziantep University, Gaziantep, Turkey

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Main Points

- The type of ligation did not appear to affect the plaque and GI values.
- *S. mutans* colonization showed variations in low-friction elastomeric ligatures, independent of surface roughness.
- Only ring-shaped low-friction elastomeric ligatures were similar to the steel ligature in terms of *S. mutans* colonization.

ABSTRACT

Objective: To compare *Streptococcus mutans* colonization between low-friction elastomeric ligatures and to correlate microbial colonization levels with the surface roughness status.

Methods: The study included 160 premolars of 10 patients. During the study period, which consisted of 4 sessions each lasting 4 weeks, the ligature types Slide™ Low-Friction Ligature (Leone, Firenze, Italy), Tough-O Energy™ (Rocky Mountain Orthodontics, Denver, USA), and Sili Ties™ (Dentsply Sirona, Surrey KT13 0NY, UK), and steel ligatures (American Orthodontics, Sheboygan, USA) as a control, were fixed to the premolar teeth by clockwise rotation among the jaw quadrants. The plaque index (PI) and gingival index (GI) were obtained before bonding (T0), 6 weeks after bonding (T1), and subsequently every 4 weeks (T2, T3, T4). Presence of *S. mutans* was analyzed by real-time polymerase chain reaction at T1, T2, T3, T4. Surface roughness was evaluated with Atomic Force Microscopy (AFM) before ligation (Ra0) and after (Ra1) ligation. The paired t-test, ANOVA, repeated measures of ANOVA, and the Kruskal–Wallis test were used for the statistical analysis.

Results: *S. mutans* colonization was significantly higher on the Slide group ($P < .05$). The lowest Ra0 was seen in Slide and the highest was seen in the Tough-O Energy group. There was no correlation between *S. mutans* colonization and Ra1 parameters of elastomeric groups ($P > .05$).

Conclusion: *S. mutans* colonization showed variations in low-friction elastomeric ligatures independent of surface roughness. Ring-shaped low-friction elastomeric ligatures were not different from the steel ligature in terms of *S. mutans* colonization.

Keywords: Real-time polymerase chain reaction, atomic force microscopy, microbiology, surface roughness, low-friction elastomeric ligatures

INTRODUCTION

During orthodontic treatment, maintaining oral hygiene becomes difficult due to the placement of bands, brackets, and ligatures in the oral cavity.[1] It was previously reported that permanent orthodontic treatment led to dense plaque formation and an increase in cariogenic and periodontal bacterial growth.[2] Throughout treatment, the presence of plaque at the gingival border was accepted as the main etiological factor in periodontal diseases, whereas increased plaque accumulation around orthodontic brackets is known to result in white-spot lesions

Corresponding author: Aysegul Gulec, e-mail: ayseguldt@yahoo.com

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and in severe cases of tooth decay, which negatively affect the quality of life.[3,4] Enamel demineralization, which results in white-spot formation, is observed due to the increase in the number and volume of acid-producing bacteria, and the decrease in pH because of the glucose metabolized by these cariogenic bacteria.[5,6] *Streptococcus mutans* is one of the bacteria that play an important role in the onset of carious lesions.[7]

Current product development efforts have resulted in the development of low-friction elastomeric ligatures to reduce the friction of orthodontic sliding mechanics.[8] The efforts to reduce friction between orthodontic wires and braces have played a role in the development of elastomeric ligatures with altered surface structures. A difference in the colonized bacteria around the brackets can be anticipated in connection with this altered surface structure. The effect of different ligation methods on microbial colonization has been a topic that researchers have been working on for a long time, but bacterial colonization on low-friction elastomeric ligatures was investigated in relatively few studies, where Slide™ elastomeric ligatures as low-friction elastomeric ligatures were compared with conventional elastomeric ligatures using microbial culture techniques.[9-14] Nowadays, the number of elastomeric ligatures showing low friction has increased in the market. However, there are no studies comparing different low-friction elastomeric ligatures concerning microbial colonization. Thus, the aim of this study was to compare *S. mutans* colonization among 3 different low-friction elastomeric ligatures that are available commercially—Slide™ Low Friction Ligature (Leone, Firenze, Italy), Tough-O Energy™ (Rocky Mountain Orthodontics, Denver, USA), and Sili Ties™ (Dentsply Sirona, Surrey KT13 0NY, UK)—with steel ligatures (American Orthodontics, Sheboygan, USA) as a control, using real-time polymerase chain reaction (RT-PCR), as this is a simple, fast, and accurate method to identify specific bacterial species and their quantities.[15] The effect of the ligature types on periodontal status was also investigated. The secondary aim of the study was to investigate the surface structures of these ligature types via atomic force microscopy (AFM), which uses a very high-resolution scanning force microscope in which surface roughness can be detected, and to associate these surface structures with bacterial colonization.[16]

METHODS

This study was approved by the Ethics Committee of Gaziantep University, (April 26, 2017/169), and was registered in ClinicalTrials.gov: NCT04185987. The power analysis sample size determination revealed that for an alpha level of 0.05, and a power of 0.8, a minimum of 9 subjects in each group was required. (G*Power version 3.0.10, Franz Faul, Universitat Kiel, Germany). Systemically healthy patients who had permanent dentition, no dental plaque, had good oral hygiene, and who did not use antibiotics or smoke for at least 2 months before the initiation of the study were invited to participate in the study during a routine bonding visit in the orthodontics department of the Dentistry Faculty of Gaziantep University. The exclusion criteria were: absence, decay, or restoration in upper or lower premolars, and the presence of any prosthetic restorations and other orthodontic attachments except brackets, tubes, and ligature wires in the mouth. The study, which was planned to include 3 different trademarked brands of low-friction elastomeric ligatures with steel ligatures as a control simultaneously present in the mouths of the patients during the treatment process, started with 10 patients (4 female and 6 male) with a mean age of 13.58 $\pm$ 0.79 years (min:12.3; max:14.6). Informed consent was obtained from all patients and their parents. The study groups are presented in Table 1.

Patients were bonded with 22′ slot for MBT brackets (Mini Master brackets; AO, Wisconsin, USA) and 0.014 NiTi wires (TriTanium™ Wire; AO, Wisconsin, USA) were attached to the brackets via steel ligatures by the same clinician (C.D.). These 0.014″ NiTi wires were kept in place throughout the study. A 2-week time period was given to the patients for getting used to brushing, at the end of which steel ligatures were removed from the patients and all ligature groups were fixed to the brackets of the patients. The teeth to be examined were defined as left and right, upper and lower, and first and second premolar teeth. During the study period, which consisted of 4 sessions each lasting 4 weeks, all ligature types were fixed to the related premolar teeth by clockwise rotation among the jaw quadrants. Rotations were also performed between the first and the second premolars of the same region. The study design is presented in Table 2. Intraoral pictures of the patients based on the study design are shown in Figure 1. The pictures of the ligature types are shown in Figure 2.

Plaque index (PI)and gingival index (GI) were measured, prior to bonding (T0), 6 weeks after bonding (T1), and subsequently every 4 weeks (T2; T3; T4), as the clinical parameters of dental plaque accumulation.[17,18] The periodontal evaluation was carried out only on the related premolar tooth by the same trained clinician (C.D.). A total of 160 ligature samples were collected from the patients at T1, T2, T3, and T4 by the same clinician (C.D), and were kept at −80°C in a transport medium until microbial analysis. Real-time PCR analysis was performed for the investigation of the presence of *S. mutans*. DNeasy Blood & Tissue Kit(Qiagen, Hilden, Germany) was used for DNA isolation, according to the manufacturer's instructions.[19] Following extraction, forward and reverse primers (forward; 5'-CCGGTGACGGCAAGCTAA-3', reverse; 5'-TCATGGAGGCGAGTTGCA-3') of *S. mutans* (Metabion International

Table 1. Study groups

Groups	Type of elastomeric ligature	Manufacturer	*N*
I	Slide™ Low-Friction Ligature	Leone, Firenze, Italy	40
II	Tough-O Energy™ Ligature	Rocky Mountain Orthodontics, Denver, USA	40
III	Sili Ties™ Ligature	Dentsply Sirona, Surrey KT13 0NY, UK	40
IV	Twisted End Steel Ligature	American Orthodontics, Sheboygan, USA	40

Table 2. Study design

Time period	Tooth number	Ligature type	Time period	Tooth number	Ligature type
2-6 weeks after bonding	15	Group I	6-10 weeks after bonding	14	Group IV
	25	Group II		24	Group I
	35	Group III		34	Group II
	45	Group IV		44	Group III
10-14 weeks after bonding	15	Group III	14-18 weeks after bonding	14	Group II
	25	Group IV		24	Group III
	35	Group I		34	Group IV
	45	Group II		44	Group I

AG Planegg, Germany) were designed and provided to investigate the presence of the bacteria and determine the bacterial load within the isolated eluates. The ATCC 25175 and ATCC 35668 strains of *S. mutans* were used as positive controls. The standards were optimized for usage in the RT-PCR study. Primarily, a master mix was prepared with RT2 SYBR Green PCR master mix kit (Qiagen, Hilden Germany) for RT-PCR. For each sample, a mixture was prepared with 12.5 µL RT2 SYBR Green PCR master mix, 0.5 µL forward primer, and 0.5 µL reverse primer 6 5 µL H_2O. The template isolated from the 5 µL samples (sample DNA) was added on this master mix prepared, the PCR tubes were capped, and RT-PCR analysis was performed using the Rotor-Gene Q instrument (Hilden Germany).

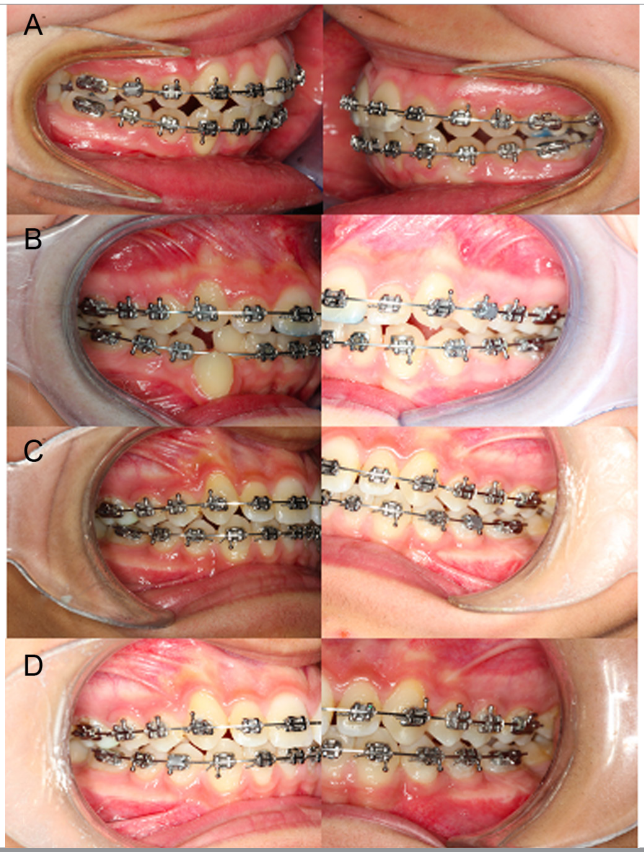

Figure 1. The intraoral pictures of the patients based on the study design. (A) First month, (B) Second month, (C) Third month, and (D) Fourth month

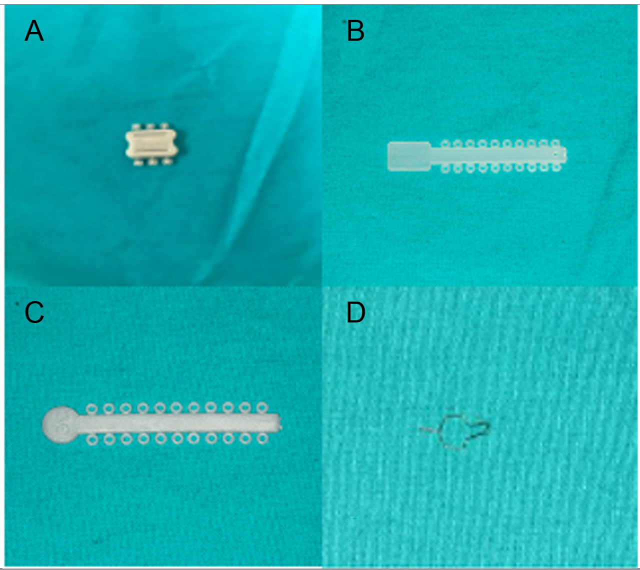

Figure 2. Ligature groups. (A) Slide™ Low-Friction Ligature (B) Tough-O Energy™ Ligature (C) Sili Ties™ Ligature (D) Twisted End Steel Ligature

For surface roughness analysis, three-dimensional surface roughness (R_a) of the 3 different types of elastomeric ligatures was analyzed by AFM (Bruker, Santa Barbara, CA) with its own specific software Nanoscope™ version-5.31R1. R_a represents the arithmetic mean of the absolute values of the profile of the scanned surface in micrometers (µm), (40 µm × 40 µm). The baseline forms of the elastomeric ligatures (R_a0) and the forms after 4-weeks of use (R_a1) were presented separately, and the surface roughness was measured on 6 different regions and calculated in nanometers.

STATISTICAL ANALYSIS

Compliance of the data with normal distribution was tested using the Shapiro–Wilk test. The comparison of the non-normally distributed data between more than 2 independent groups was performed using the Kruskal–Wallis test, and all pairwise multiple comparison tests and normally distributed data were analyzed using one-way analysis of variance (ANOVA). The comparison between 2 different time points was performed using the paired samples *t*-test, and between more than 2-time points was performed using repeated measures of ANOVA and LSD multiple comparison tests. The correlations between surface roughness and microbial colonization were tested using Spearman's correlation coefficient. The Statistical Package for Social Sciences version 22.0 software (IBM Corp.; Armonk, NY, USA) was used for statistical analysis. A *P* value of <.05 was considered significant.

RESULTS

Descriptive statistical data on time-dependent means of PI and GI values and inter-group comparisons are presented in Table 3. PI and GI values at all-time points during the treatment were higher compared to the ones at baseline in all groups ($P < .001$).

Table 3. Descriptive statistical data on time-dependent means of PI and GI values and inter-group comparisons

	Group	*n*	T0 Mean±SD	T1 Mean±SD	T2 Mean±SD	T3 Mean±SD	T4 Mean±SD	*P* within groups
Plaque index (PI)	I	10	0.44 ± 0.19[abcd¥]	2.20 ± 0.36	2.25 ± 0.33	2.39 ± 0.26	2.37 ± 0.20	<.001[€]
	II	10	0.56 ± 0.1 [abcd¥]	2.33 ± 0.23	2.32 ± 0.11	2.48 ± 0.13	2.28 ± 0.12	<.001[€]
	III	10	0.40 ± 0.22 [abcd¥]	2.19 ± 0.36	2.33 ± 0.76	2.25 ± 0.08	2.27 ± 0.20	<.001[€]
	IV	10	0.46 ± 0.09 [abcd¥]	2.25 ± 0.45	2.18 ± 0.48	2.14 ± 0.09	2.19 ± 0.26	<.001[€]
	P between groups*		NS	NS	NS	NS	NS	
Gingival index (GI)	I	10	0.58 ± 0.23 [abcd¥]	2.09 ± 0.54	2.19 ± 0.33	2.22 ± 0.31	2.15 ± 0.49	<.001[€]
	II	10	0.53 ± 0.18 [abcd¥]	2.14 ± 0.64	2.30 ± 0.67	2.28 ± 0.40	2.20 ± 0.54	<.001[€]
	III	10	0.60 ± 0.33 [abcd¥]	2.51 ± 0.78	2.50 ± 0.49	2.48 ± 0.13	2.35 ± 0.87	<.001[€]
	IV	10	0.48 ± 0.45 [abcd¥]	2.01 ± 0.66	2.25 ± 0.92	2.12 ± 0.18	2.11 ±0.73	<.001[€]
	P between groups*		NS	NS	NS	NS	NS	

*ANOVA; [€]Repeated measures of ANOVA; [¥]LSD multiple comparison test.
T0, Prior to bonding; T1, 6 weeks after bonding; T2, T3, T4 subsequently every 4 weeks; $P \leq$,05;.
[a]significantly different from T1, [b]significantly different from T2, [c]significantly different from T3, [d]significantly different from T4.
SD, standard deviation.

Table 4 displays the total count of *S. mutans* colonization on the ligature surfaces and intergroup comparisons. *S. mutans* colonization on Group I ligature was significantly higher compared to all other groups ($P = .016$). No significant difference was observed between other elastomeric ligature groups or between these groups and the control group ($P > .05$).

Table 5 shows the intergroup and intragroup comparisons of surface roughness analysis. According to R_a0 values, the lowest roughness was seen in Group I and the highest roughness was seen in Group II. When the R_a1 values of all groups were compared, the lowest roughness was observed in Group III, and the highest roughness was observed in Group II. Intra-group comparisons of R_a0 and R_a1 revealed that the R_a1 of Group I was significantly higher compared to the R_a0 ($P = .012$), whereas no statistically significant difference was found between Group II and Group III ($P > .05$, Figure 3).

There was no correlation between the total *S. mutans* colonization and R_a1 parameters of the elastomeric ligature groups ($P > .05$) (Table 6).

DISCUSSION

The literature clearly shows that fixed orthodontic treatment increases plaque formation, bacterial colonization, and enamel

Table 4. Total count of *S. mutans* colonization on the ligature surfaces and inter-group comparisons

S.mutans colonization	Groups: Group I n = 40 Mean ± SD	Group II n = 40 Mean ± SD	Group III n = 40 Mean ± SD	Group IV n = 40 Mean ± SD	*P* (within groups)	*P* (between groups): I-II	I-III	I-IV	II-III	II-IV	III-IV
	10.8± 3.74	6.65 ± 2.81	6.09± 2.47	5.85± 3.02	.016[£]	.025*	.008*	.005*	.674	.553	.863

*All pairwise multiple comparison tests; [£]Kruskal–Wallis test.
SD, standard deviation.

Table 5. Intergroup and intragroup comparisons of surface roughness analysis

	Groups: Group I n = 6 Mean ± SD	Group II n = 6 Mean ± SD	Group III n = 6 Mean ± SD	*P* (between Groups): *P*	I-II	I-III	II-III
R_a0 (µm)	0.06 ± 0.01	0.21 ± 0.03	0.1 ± 0.02	.001[€]	0.001[β]	0.004 [β]	0.001[β]
R_a1(µm)	0.17 ± 0.05	0.23 ± 0.01	0.1 ± 0.03	.001[€]	0.026[β]	0.046 [β]	0.010[β]
P (within groups)	.012*	0.412	0.765				

*Paired *t*-test; [€]Repeated measurements of ANOVA; [β]LSD multiple comparison test; $P \leq .05$.
SD, standard deviation.

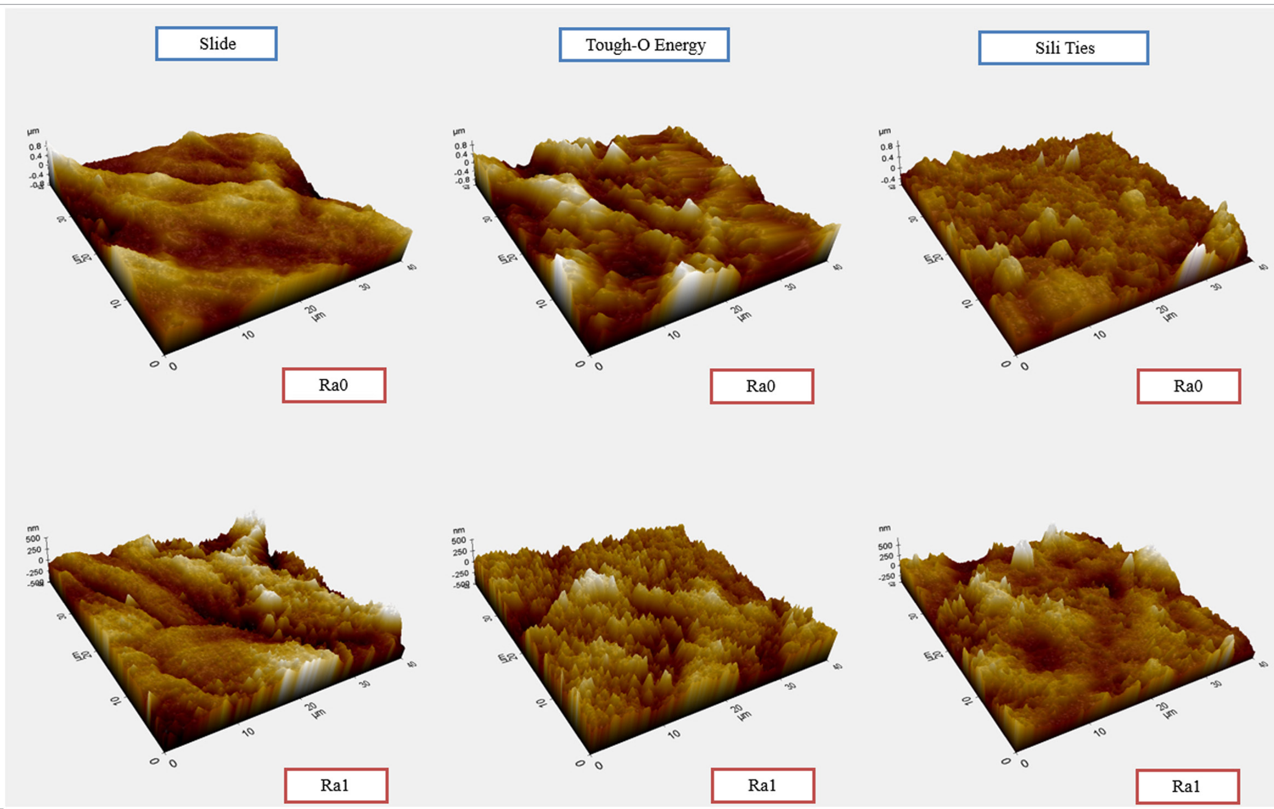

Figure 3. AFM images of ligature groups

Table 6. Correlation between the total *S. mutans* colonization and R_a1 parameters of elastomeric ligature groups

	Group I		Group II		Group III	
S mutans	*P*	Correlation coefficient	*P*	Correlation coefficient	*P*	Correlation coefficient
	.623	-0.257	.111	−0.714	.704	0.200

Spearman's rank correlation analysis.

decalcification.[5] Many studies have investigated the effects of ligation techniques on dental plaque retention and microbial flora, but only a few focused on the low-friction elastomeric ligatures; none of them investigated the surface properties of elastomeric ligatures as an additional factor.[12-14,20,21] To meet the deficit to some extent, 3 different low-friction elastomeric ligatures were compared in the present study in terms of microbial colonization, periodontal status, and surface morphology.

Three different commercially available brands of elastomeric ligatures as low-friction ligatures made up the material of this study. Slide™ is a product that is manufactured with a special polyurethane mix by injection molding. Although its application is similar to that of conventional elastic ligatures, the shape of the ligature is rather bulky. Once ligated on the bracket, it creates a passive ligation on the slot with a lower frictional force that leaves the archwire free to slide. Tough-O Energy™ and Sili Ties™ ligatures are ring-shaped, in the same way as conventional elastomeric ligatures, but less frictional force occurs between the archwire and ligatures due to their production techniques, which is the distinguishing property of these 2 elastomeric ligatures. Based on the study design, 3 different brands of elastomeric ligatures, as well as steel ligatures as a control group, were present in the mouth at the same time. Together with the advantage of keeping the number of participating subjects relatively low, this design reduced the duration of follow-up and minimized the possible hygiene motivation loss of the patient. Clockwise rotation of the ligature groups around the jaw quadrants at each control visit aimed to prevent the brushing habits of the patient from affecting the results. The aim of the rotation between the first and the second premolars in the same region was to avoid the possible effects of microorganisms remaining from the previous session.

PI and GI measurements used for the evaluation of periodontal health revealed lower values before bonding compared to all other measurements. This finding is consistent with those of previous studies reporting that the orthodontic fixed treatment increased plaque accumulation.[11,22,23] There was no statistically significant difference between groups at any of the time points, showing that the oral hygiene motivation of the patients remained stable during the study.

In the present study for microbial evaluation, RT-CR, which can detect a small number of cariogenic bacteria in patients who are likely to experience enamel demineralization, was used. The number of studies evaluating the effects of ligation methods on microbial flora using PCR is quite low.[24,25] To eliminate the difficult and time-consuming laboratory procedures of culture techniques, the highly sensitive RT-PCR technique was preferred.

A consortium of multiple microorganisms acts collectively, and possibly synergistically, to initiate and expand the caries lesion.[26] S. *mutans* is not the sole cause of caries lesions, but is still a frequently investigated microorganism because it plays an important role in the onset of these lesions.[11,27] Total *S. mutans* counts showed that microbial accumulation on the Slide™ elastomeric ligatures was higher compared to all other groups of ligatures. In the study of Bhagchandani et al.,[12] using culture techniques for microbial examination, 4 different types of ligatures and steel ligature as a control were compared in terms of microbial colonization. The highest microbial colonization was observed on Slide™ elastomeric ligatures, similar to our study. Investigators have concluded that this may be due to the complicated, rough, and high-volume structure of those groups of ligatures. In another study, Akgün et al.[13] compared Slide™ elastomeric ligatures and conventional elastomeric ligatures concerning aerobic and anaerobic bacterial growth and plaque accumulation using microbiological culture methods, and observed no difference. The comparison of low-friction elastomeric ligatures between themselves rather than with conventional elastomeric ligatures and the bacteria type examined reveal the difference of this study from that of Akgün et al.[13]

Interestingly, no difference was observed between the ring-shaped low-friction elastomeric ligature types, or between these groups and the steel ligature control group, with regard to *S. mutans* count. This finding is compatible with the findings of the study of Türkkahraman et al.[11] comparing conventional ring-shaped elastomeric ligatures and steel ligatures in terms of microbial colonization using culture techniques, which revealed no significant difference between these 2 types of ligatures but a higher number of microorganisms on elastomeric ligatures. However, in many studies in the literature, elastomeric ligatures have been reported to have more microbial colonization than steel ligatures.[28-30] In contrast to the current knowledge, microbial colonization on the surfaces of a new group of ligatures, low-friction elastomeric ligatures, was assessed in the present study. Within its limitations, this is the most important aspect in which this study contributed to literature. The fact that no difference was observed between ring-shaped low-friction elastomeric ligatures and steel ligatures in terms of microbial colonization could lead to these types of ligatures being preferred more.

In our study, the surface properties of low-friction elastomeric ligatures were measured by AFM at baseline and after usage. The measurement of surface roughness via AFM was also previously performed in orthodontics.[31] When the baseline surface structures of all groups were compared, the lowest roughness was observed in Slide™ ligatures and the highest roughness was observed in Tough-O Energy™ ligatures. After usage, AFM analysis revealed that the greatest change in surface roughness was observed in the Slide group, and the other elastomeric ligature groups showed an insignificant change in surface roughness. Condo et al.[32] investigated the morphological changes observed in the structures of conventional elastomeric ligatures and Slide™ elastomeric ligatures using scanning electron microscope (SEM), both before and after usage. The study reported a significant difference in the internal and external diameters and the thicknesses of conventional elastomeric ligatures. They reported morphological irregularities in the sizes of Slide™ ligatures. The authors concluded that wing lengths were increased due to the effect of elastomeric deformation observed at the wings of Slide™ ligatures, which might have resulted in impaired contact between the wire and the ligature. The possibility of statistical evaluation as a result of the measurements performed by AFM allowed us to obtain more quantitative results compared with those studies conducted using SEM.

One of the parameters to be investigated in the present study was the correlation between the surface roughness of elastomeric ligatures and bacterial growth. No correlation was observed between the total *S. mutans* count observed on the surface of the elastomeric groups and R_a1 parameters. In the study by Guimares et al.[33] investigating the surface and mechanical properties of elastomeric ligatures, surface roughness analyses were performed on SEM images. According to the results of their study, the amount of change observed in surface roughness was more than the amount of change observed in mechanical properties. In the study where surface roughness was not evaluated statistically, this increase observed in roughness was concluded to have been caused by plaque accumulation. Although no significant correlation was observed in the present study either, it was found that more microorganisms accumulated on elastomeric ligatures in proportion to the increase in roughness.

Together with the periodontal status and surface structures, this study provides information on the quantitative analysis of *S. mutans* in patients using low-friction elastomeric ligatures. The ring-shaped low-friction elastomeric ligatures did not differ from the steel ligature in terms of *S. mutans* colonization. Keeping in mind that no relation could be established between elastomeric ligature surface roughness and *S. mutans* count, a feasible explanation for this result may be the dimensional differences of the ligatures. Slide™ ligatures may have more *S mutans* due to their unique and bulky shape. The main limitations of this study are the limited number of patients, bacteria investigated, and surface roughness samples, due to the financial constraints.

CONCLUSION

According to our findings with the limitations of in vivo studies:

- Plaque and gingival index values do not appear to be affected by ligation type.
- Slide™ ligatures demonstrated a higher quantity of *S. mutans* colonization compared to other low-friction ligatures.
- No difference was observed between Tough-O Energy™ and Sili Ties™ ligatures and steel ligatures, with regard to *S. mutans* colonization.

- No correlation was observed between the surface roughness of low-friction elastomeric ligatures and the total *S. mutans* count.

Ethics Committee Approval: This study was approved by Ethics committee of Gaziantep University, (Approval No: 2017/169).

Informed Consent: Written informed consent was obtained from the patients who agreed to take part in the study.

Peer Review: Externally peer-reviewed.

Author Contributions: Concept - A.G., C.D.; Design - A.G., C.D.; Supervision - A.G.; Funding - C.D.; Materials - C.D.; Data Collection and/or Processing - C.D., F.E., M.S., M.K.; Analysis and/or Interpretation - F.E, M.S., M.K.; Literature Review - C.D., A.G.; Writing - C.D.; Critical Review - A.G.

Conflict of Interest: The authors have no conflict of interest to declare.

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