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Original Article

Dentistry Section

An Examination of Bacterial Colonisation on Nickel-Titanium Arch-wires with Different Surface Properties

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ABSTRACT

Introduction: Microbial dental plaque accumulation during fixed orthodontic treatment, leads to iatrogenic decalcification of the enamel and to development of the white spot lesions. The surface charcteristics of orthodontic wires can affect this situation.

Aim: To comparatively assess the uncoated nickel-titanium archwires (A-NiTi) and epoxy resin-coated nickel-titanium archwires (A-NiTi/ER) *in vivo*, regarding the periodontal status, *Streptococcus mutans* (SM) and *Lactobacillus* (LB) colony numbers on wires and in saliva samples.

Materials and Methods: In this cross-sectional study, 31 individuals (20 females, 11 males) with a mean age of 15.5±1.84 years were included. A-NiTi and A-NiTi/ER were applied randomly and in sequential order. Saliva and archwire samples were obtained, to assess the amount of SM and LB colony numbers before archwire application, and after archwire applications (A-NiTi and A-NiTi/ER). Periodontal parameter that was assessed was gingival bleeding index. Factorial design repeated measures ANOVA and Bonferroni methods were used for statistical analysis.

Results: The SM count in saliva before archwire application was statistically lower than A-NiTi and A-NiTi/ER application ($p<0.001$) statistically significant differences were found between A-NiTi and A-NiTi/ER archwires for LB colony numbers ($p<0.001$). The LB counts between saliva and archwires for A-NiTiand A-NiTi/ER, were in a moderate positive relationship with each other. No significant difference in the GBI percentage was observed ($p>0.05$).

Conclusion: Increased LB colony numbers were obtained on A-NiTi/ER compared to A-NiTi. The increase in LB colony numbers between saliva and archwires for A-NiTi, and similarly for A-NiTi/ER were correlated with each other.

Keywords: Lactobacillus, Orthodontics, Orthodontic wires, Preventive, *Streptococcus mutans*

INTRODUCTION

It is important to maintain aesthetic appearance and oral health while obtaining optimal occlusion and function with fixed orthodontic treatment. However, under fixed orthodontic treatment, a rapid change in the bacterial composition of the dental plaque with and an increased presence of cariogenic bacteria, particularly, *Streptococcus mutans* (SM) and *Lactobacillus* (LB) species have been observed, after applying the orthodontic attachments [1,2]. Microbial dental plaque accumulation during fixed orthodontic treatment, leads to iatrogenic decalcification of the enamel and to the development of white spot lesions, which are the precursors of caries [3]. Orthodontic attachments produced from materials that reduce microbial dental plaque accumulation and provide aesthetic appearance, can both maintain the oral health and preserve the aesthetic appearance [4].

Recently, increased aesthetic concerns during fixed orthodontic treatment have led to the production of orthodontic attachments in tooth colour. Owing to surface coating technology that enables the development of aesthetic characters of metallic arch wires, these wires can be coated with materials, such as palladium polymer, polytetrafluoroethylene/Teflon, and Epoxy Resin (ER), which are in tooth colour [5-8]. It was shown that coating technology improves not only aesthetic characteristics of the wire but also mechanical properties as well. Through this surface modification, surface roughness and friction force reduce, and resistance to corrosion increases, by decreasing Ni release [5,9-11]. *In vitro* and *in vivo* studies reported that when the orthodontic archwires are coated, the biofilm formation has been inhibited by acquiring anti-adhesive properties [4,12]. Thus, the accumulation of bacterial plaque has decreased. However, noticeable peeling of the coated archwires under *in vivo* conditions during fixed orthodontic therapy may cause bacterial accumulation on peeled surfaces of coated archwires [13,14]. If white spot lesions occur by the effect of increased cariogenic flora, due to the accumulation of microbial plaque on the peeled surfaces of aesthetic archwires, neither the patient needs can be satisfied by using aesthetic wires nor the successful treatment outcome can be achieved. However, in literature very less and conflicting information exists about this issue in-vivo studies [4,13,14]. Therefore, more in vivo studies are required to evaluate the microbiological properties, such as bacterial adhesion characteristics of coated archwires.

This study comparatively assessed the effect of surface properties of uncoated nickel-titanium archwires (A-NiTi) and epoxy resin-coated nickel-titanium archwires (A-NiTi/ER), under in vivo conditions for gingival bleeding index as a periodontal health parameter and SM and LB colony numbers, on the wire and in saliva samples. Additionally, the correlation between SM and LB counts in Saliva and both the arch wires was assessed. The null hypothesis was that there was no difference in SM and LB colony numbers on the wires and in the saliva during the sequential application of A-NiTi and A-NiTi/ER, in the same individuals.

MATERIALS AND METHODS

The present study was single-blind, prospective study with a cross-over design which was approved by Süleyman Demirel University, Faculty of Medicine, Clinical Research Ethics Committee (9.07.2015/145). It was performed in the Department of Orthodontics, Faculty of Dentistry, and in the Department of Microbiology, Faculty of Medicine, Süleyman Demirel University, Isparta, Turkey. The study was performed between the September 2015 to October 2016.

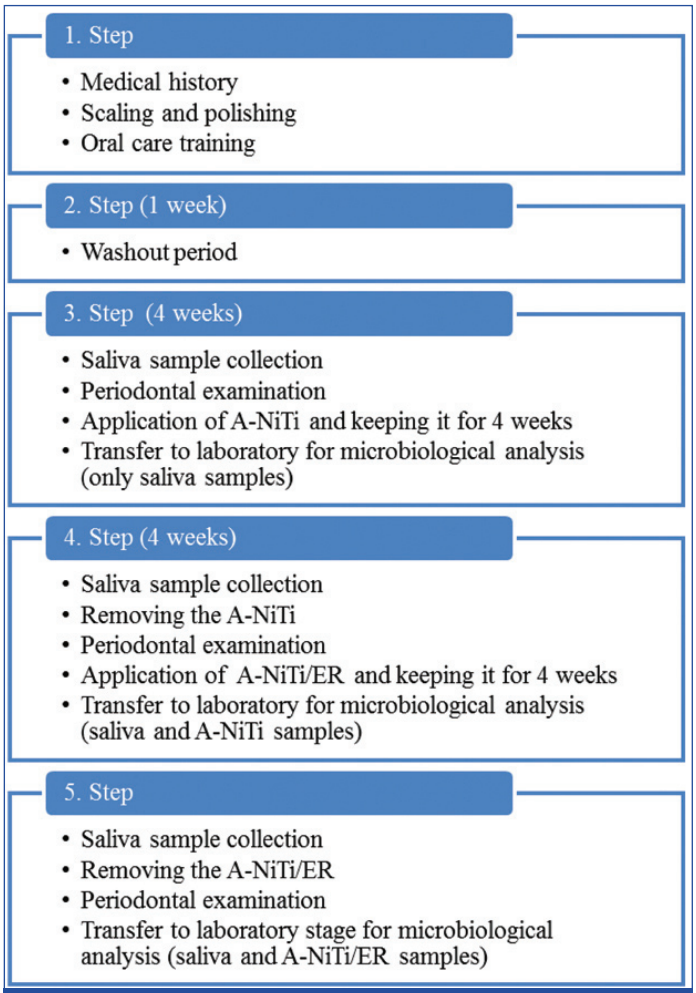
Written informed consent form was obtained from all the subjects. The inclusion criteria for participants were: 1) patients under non-extraction fixed orthodontic treatment for at least 12 months; 2) in

finishing phase; 3) brackets present on all six teeth in each of the four quadrants; 4) periodontally healthy; 5) having permanent dentition; 6) systemically healthy; 7) over 12 years of age; 8) no prosthetic restorations; 9) no other attachments, except brackets or bonded molar tubes in mouth; 10) no caries; 11) no antibiotic usage and no fluoride application for at least 1 month; 12) no regular mouth-wash usage; and 13) no other orthodontic treatment. G*Power version 3.0.10 (Franz Faul Universität, Kiel, Germany) program was used to determine the number of individuals to be included in the study according to literature [4], and the power of the study was calculated as 95%. The sample size thus calculated was 30 patients. For the application sequence of archwires, simple random sampling was used. Thirty-one patients (20 female, 11 male), under fixed orthodontic treatment with a 0.018. Roth metal bracket system for at least 12 months, with the mean age of 15.5±1.84 years, who fulfilled the study criteria, were included [Table/Fig-1]. In this study, two different archwires with distinct surface characteristics were applied randomly and in sequential order. Participants were not informed about the surface features of the archwires.

Gender	N	$\bar{X}$±SD	Min	Max
Female	20	15.52±1.88	12.1	18.5
Male	11	15.47±1.97	12.1	17.3
Total	31	15.50±1.84		

[Table/Fig-1]: Age and gender distribution of the study group.
N: Number; $\bar{X}$: Mean; SD: Standard deviation; Min: Minimum; Max: Maximum

[Table/Fig-2] explains the study steps. In oral hygiene training, the modified Bass technique was explained, and oral hygiene motivation was done. Additionally, an oral care set, including manual tooth brush (Oral-B Ortho®, P&G, USA), interdental brush (Oral-B Pro Expert Clinic Line Interdental Kit®, P&G, USA) and tooth paste (Colgate Triple Action®, Colgate-Palmolive Coop., China) was given to all participants, for their thrice daily oral care. Before the collection of saliva samples, individuals brushed their teeth before bedtime, one day prior to each appointment, and arrived in the morning without breakfast and brushing. Unstimulated saliva was collected, by seating the subject in an upright position at rest, tilting the head forward and draining to a sterile container for 10 minutes. Gingival Bleeding Index (GBI) was the periodontal parameter that was evaluated in this study [15]. In the washout period, all attachments, except brackets and molar tubes, were removed for 1 week. Individuals were requested to perform their oral care with the given oral care set. After 1 week of washout period, 0.016×0.022´ diameter A-NiTi (upper-lower) (NiTi Memory Wire American Orthodontics, Sheboygan, WI, USA) or A-NiTi/ER (upper-lower) (NiTi EverWhite Cosmetic, American Orthodontics, Sheboygan, WI, USA) were applied, according to the selection by lot. Two opaque envelopes, each one containing an archwire with different surface characteristics, were prepared and one was chosen by the patient. Then, the disinfected archwires were applied to both upper and lower dental arches of each patient, via elastic ligatures. The duration of archwire application (upper-lower) was 4 weeks. Before removing the archwire 4 weeks later, individuals brushed their teeth before bedtime, one day prior to appointment, and arrived in the morning without breakfast and brushing. At this appointment, the lower and upper archwires were divided into three parts, by using a sterile distal cutter from the mesial of the right and left canine teeth and these were removed without any contact to the cheek mucosa and the lip. Next, the other archwire (upper-lower), with different surface characteristics, was applied. Prior to the placement of the next wires, brushing was performed. At each time, saliva samples from individuals were collected in to a sterile empty container. Also, samples of the archwires were placed into a sterile container, containing Phosphate Buffered Saline solution (PBS) and the upper and lower archwires were stored in different containers. Both samples were transferred to the Medical Microbiology Laboratory of Süleyman Demirel University, Medical Faculty within 1 hour. After one week washout period, each arch wire had been applied for 4 weeks. The total duration of this in vivo study was 9 weeks.



[Table/Fig-2]: Study steps.

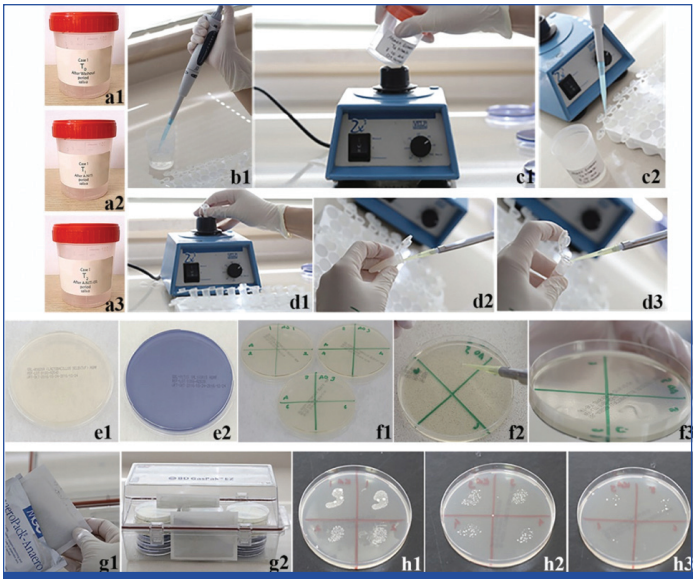
Microbiological Analyses of Saliva and Archwire Samples

The saliva samples, transferred in a sterile container, were homogenised in a vortex mixer (Velp Scientifica, Fisher ZX3 Vortex Mixer, Italy) and 10-fold serial dilutions of 10^{-1} to 10^{-10} were prepared in sterile 0.9% NaCl isotonic solution, by taking 1 mL of saliva. After 10-fold serial dilutions, 10-µL saliva aliquots were plated in duplicate, onto Mitis-Salivarius Agar for SM culture and Rogosa Lactobacillus Selective Agar for LB culture (GBL, Istanbul, Turkey). Incubation of the samples were performed in an anaerobic atmosphere (AnaeroPack®-Anaero, Mitsubishi Gas Chemical Co. Inc., Japan) at 35±2°C for 48 hour. After incubation, the total number of Colony-Forming Units (CFU) on each plate was counted [Table/Fig-3].

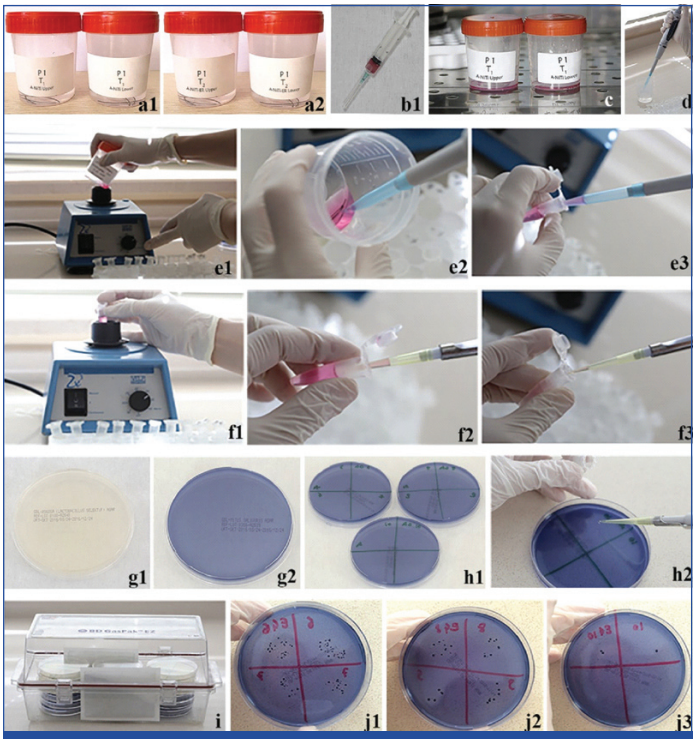
Archwire samples were delivered to the laboratory in a sterile tube containing PBS. PBS was removed from the tube using a sterile syringe, without touching the wire samples and 3 mL of 0.25% trypsin-EDTA solution was added to the tube. The archwires were kept in this solution at 37°C for 45 minutes. Then, each specimen was homogenised in a vortex mixer. After homogenization, 1 mL of 0.25% trypsin-EDTA solution, covering the archwire sample, was taken for microbiological cultivation procedure. Next, microbiological cultivation was performed in the same manner as it was for saliva samples [Table/Fig-4].

SM and LB Colony Counts in Saliva and Archwire Specimens

SM and LB colony counts were determined, according to the dilution ratios of the plates on which the colonies were counted with the naked eye. Duplicate inoculation of the plates was performed for each dilution. Hence, the number of colonies on the plates of



[Table/Fig-3]: Microbiological analyses of saliva.
a1-3, saliva samples of a case; **b1**, elnen sterile eppendorf tubes were prepared from 0 to l0 for each sample. 90 µL of 0.9% NaCl isotonic solution was added to each of the other 10 tubes, except one tube which was stayed empty; **c1**, saliva samples were homogenised in a vortex mixer for 2 min. **c2**, one mL of saliva sample was taken to the empty eppendorf tube; **d1**, one mL of homogenised saliva sample was re-homogenised in vortex mixer for 20 sec; **d2-3**, 100 µL of saliva was transferred to tube #1 with a sterile pipette from this 1 mL sample. The total amount of liquid in the tube was completed to 1ml 10^{-1} dilution was obtained, 10-fold serial dilutions of 10^{-1} to $l0^{-10}$" were prepared in sterile 0.9% NaCl isotonic solution; **e1**, Rogosa Lactobacillus Selective Agar medium; **e2**, Mitis-Salivarius Agar medium; **F1-3**, 10^{-1} µL saliva aliquots were plated in duplicate, onto Mitis-Salivarius Agar for SM culture, Rogosa Lactobacillus Selective Agar for LB culture; **g1,2**, incubation of the samples was performed in an anaerobic atmosphere; **h1-3**, after incubation, the plates according to their dilution fold was sorted and the total number of colony-forming units (CFU) were counted



[Table/Fig-4]: Microbiological analyses of wires.
a1,2, archwire samples in a sterile tube containing buffered saline solution (PBS); **b1,** 3 mL of 0.25% trypsim-EDTA solution; **c,** PBS was removed from the tub using a sterile syrings, without touching the wire samples and 3 mL of 0.25% trypsin-EDTA solution was added to the tube, the archwires were kept In this solution at 37°C for 45 min; **d;** eleven sterile eppendorf tubes were prepared from 0 to 10 for each samples. 900 µL of 0.9% NaCl isotonic solution was added to each of the other 10 tubes, except one tube which was stayed empty; **e1**, each specimen was homogenised in a vortex mixer for 2 min; **e2,3**, one mL of 0.25% Trypsin-EDTA solution, including the wire samples was taken into tube #0 (empty) with a sterile pipette; **f1,** this 1 mL sample was re-homogenised for 20 sec; **f2**, 100 µL of solution was then transferred to tube #1 with a sterile pipette; **f3**, the total amount of liquid in the in the tube was completed to 1 mL and 10^{-1} dilution was obtained, than 1-fold serial dilutions were prepared in sterile **g2**, Mitits-Salivarius Agar medium; **h1,2**, 10-µL saliva aliquots were plates in duplicate, onto Mistis-Salivarius Agar for SM culture, Rogosa Lactobacillus Selective Agar for LB culture; i, incubation, of the samples was performed in an anaerobic atmosphere; **j1-3**, after incubation, the plants according to their dilution fold was sorted and the total number of colony-forming units (CFU) were counted.

countable dilution was determined, by taking the arithmetic average of the two cultures. SM and LB colony counts of saliva and lower and upper archwire samples per individualwere expressed as colony forming units in 1 mL (CFU/mL) of the sample. For determining the SM and LB colony numbers found in 1 mL sample, the number of colonies detected on the plate was multiplied by the plate dilution factor and divided by the volume transferred from the dilution tube to the culture plate.

$$\text{CFU/mL} = \frac{(\text{colony count} \times \text{dilution factor})}{\text{volume transferred from dilution tube to the culture plate (mL)}}$$

Dilution factor=1/dilution ratio

The original data measured in CFU were transformed to log 10 for statistical analysis and reported as log CFU [16].

In the present study, saliva samples and gingival bleeding index were obtained after the washout period before archwire application, 4 weeks after the application of A-NiTi, and 4 weeks after the application of A-NiTi/ER. Archwire samples were obtained 4 weeks after the application of A-NiTi and 4 weeks after the application of A-NiTi/ER for both upper and lower dental arches, separately. Microbiological counts including SM and LB colony numbers were determined in saliva and archwire samples. Periodontal tissue parameters consisted of GBI.

STATISTICAL ANALYSIS

SPSS program (SPSS Statistics 20.0, Chicago, USA) was used. Normal distribution of data was determined by Shapiro-Wilks and Box's M tests. The data obtained in terms of SM and LB colony numbers in saliva were analysed by factorial design repeated measures ANOVA after log transformation. Data of the bleeding index evaluated in patients were also analysed by factorial design repeated measures ANOVA. In the analyses, the time factor had three levels, before archwire application, after A-NiTi application, after NiTi/ER application. The microbiological data obtained in terms of SM and LB numbers of the arch wires were analysed with factorial design repeated measures ANOVA after log transformation. In the analysis, the time factor had two levels, after A-NiTi application, after NiTi/ER application; the jaw factor had two levels, the upper and lower jaw. Repeated measurements were performed at both factor levels. Bonferroni method was used to compare the differences between time intervals. The significance level was assessed as 0.05. Pearson correlation test was used to determine the relationship between bacteria counts of saliva and arch wires.

RESULTS

For SM number in saliva, the difference between baseline and A-NiTi; and baseline and A-NiTi/ER was statistically significant ($p<0.001$). There were no statistically significant differences between the mean values of LB colony numbers in saliva samples ($p>0.05$) [Table/Fig-5]. For GBI index, there were no statistically significant differences between the mean values of the time intervals ($p>0.05$) [Table/Fig-5].

There were no significant arch*archwire interactions between upper and lower dental archwires according to the statistical analyses of the mean values of SM or LB colony numbers determined in the samples of archwires with different surface characteristics ($p>0.05$) [Table/Fig-6]. Statistically significant differences were found between the total mean LB colony numbers of A-NiTi and A-NiTi/ER ($p<0.001$) [Table/Fig-6].

According to Pearson's correlation coefficient results, there was a moderate positive correlation between LB colony numbers in saliva and A-NiTi of the upper dental arch ($r=0.668$), and between LB colony numbers in saliva and A-NiTi of the lower dental arch ($r=0.550$) ($p<0.01$) [Table/Fig-7]. A moderate positive correlation was found between LB colony numbers in saliva and A-NiTi/ER of the upper dental arch ($r=0.562$) and between LB colony numbers

	BA ($\bar{X}$±SD)	A-NiTi ($\bar{X}$±SD)	A-NiTi/ER ($\bar{X}$±SD)	p-value	BA–A-NiTi	A-NiTi–A-NiTi/ER	BA–A-NiTi/ER
Saliva SM	10.56±1.66	11.88±1.42	11.98±1.29	0.000	***	NS	***
Saliva LB	5.76±1.39	6.24±1.50	6.31±1.65	0.122	NS	NS	NS
GBI	28.13±11.50	26.03±9.28	24.97±8.37	0.141		NS	NS

[Table/Fig-5]: Descriptive statistics and statistical evaluation of SM and LB colony numbers in saliva and gingival bleeding index value.
BA: Before archwire application; A-NiTi: After A-NiTi application; A-NiTi/ER: After NiTi/ER application; *p<0.05, **p<0.01; ***p<0.001; GBI: Gingival bleeding index; NS: Non-significant; $\bar{X}$: mean value; SD: Standard deviation; P, statistical analysis results according to factorial design repeated measures ANOVA; the Bonferroni method was used for post-hoc analysis

Colony number	Arch	A-NiTi ($\bar{X}$±SD)	A-NiTi/ER ($\bar{X}$±SD)	P (total)	P (Arch* Archwire interaction)
SM	Upper	9.64±1.27	9.58±1.13	NS	NS
	Lower	9.49±1.11	9.60±0.95		
	Total (Upper+Lower)	9.56±0.19	9.59±0.15		
LB	Upper	4.06±1.15	4.80±1.64	***	NS
	Lower	3.63±1.08	4.40±1.50		
	Total (Upper+Lower)	3.84±0.18	4.60±0.25		

[Table/Fig-6]: Descriptive statistics and statistical evaluation of SM and LB colony numbers on upper and lower dental archwire.
A-NiTi: After A-NiTi application; A-NiTi/ER: After NiTi/ER application; *p<0.05, **p<0.01; ***p<0.001; NS: Non-significant; $\bar{X}$: Mean value; SD: Standard deviation; P (Arch * Archwire interaction), statistical analysis results according to factorial design repeated measures ANOVA; P (total), statistical analysis results according to the Bonferroni method, used for post-hoc analysis

in saliva and A-NiTi/ER of the lower dental arch (r=0.591) (p <0.01) [Table/Fig-8] [17]. A moderate positive correlation was found between upper dental arch A-NiTi and lower dental arch A-NiTi, regarding SM colony numbers (r=0.570), and there was moderate positive correlation between upper dental arch A-NiTi and lower dental arch A-NiTi, regarding LB colony numbers (r=0.659)(p<0.01) [Table/Fig-9]. A low positive correlation was found between upper dental arch A-NiTi/ER and lower dental arch A-NiTi/ER, regarding SM colony numbers (r=0.301). Also, there was moderate positive correlation between upper dental arch A-NiTi/ER and lower dental arch A-NiTi/ER, concerning LB colony numbers (r=0.624) (p<0.01) [Table/Fig-10].

Bacteria colony number	Upper A-NiTi SM	Upper A-NiTi LB	Lower A-NiTi SM	Lower A-NiTi LB
A-NiTi saliva SM	r=-0.003	r=0.096	r=-0.148	r=0.145
A-NiTi saliva LB	r= -0.304	r=0.668**	r=-0.253	r=0.550**

[Table/Fig-7]: Correlation of SM and LB colony numbers between saliva and A-Niti.
*p<0.05; **p<0.01; ***p<0.001; r, Pearson correlation coefficient number; 0.50<r<0.70, moderate positive correlation [17]; A-NiTi, after A-NiTi application

Bacteria colony number	Upper A-NiTi/ER SM	Upper A-NiTi/ER LB	Lower A-NiTi/ER SM	Lower A-NiTi/ER LB
A-NiTi/ER Saliva SM	r=-0.084	r=-0.213	r=-0.340	r=-0.168
A-NiTi/ER Saliva LB	r=-0.077	r=0.562**	r=0.259	r=0.591**

[Table/Fig-8]: Correlation of SM and LB colony numbers between saliva and A-NiTi/ER.
*p<0.05; **p<0.01; ***p<0.001; r, Pearson correlation coefficient number; 0.50<r<0.70, moderate positive correlation [17]; A-NiTi/ER: After NiTi/ER application

Bacteria colony number	Upper A-NiTi SM	Upper A-NiTi LB	Lower A-NiTi SM	Lower A-NiTi LB
Upper A-NiTi SM		r=-0.091	r=0.570**	r=-0.182
Upper A-NiTi LB	r=-0.091		r=-0.257	r=0.659**
Lower A-NiTi SM	r=0.570**	r=-0.257		r=-0.061
Lower A-NiTi LB	r=-0.182	r=0.659**	r=-0.061	

[Table/Fig-9]: Correlation of SM and LB numbers between upper and lower dental archwires of A-NiTi.
*p<0.05; **p<0.01; ***p<0.001; r, Pearson correlation coefficient number; 0.50<r<0.70, moderate positive correlation [17]; A-NiTi: After A-NiTi application

DISCUSSION

The durability of the coating ensures the persistence of the properties acquired by the coating process. However, the friction applied by the stainless-steel ligature wire may peel the coating layer of the

	Upper A-NiTi/ER SM	Upper A-NiTi/ER LB	Lower A-NiTi/ER SM	Lower A-NiTi/ER LB
Upper A-NiTi/ER SM		r=-0.174	r=0.301**	r=0.002
Upper A-NiTi/ER LB	r=-0.174		r=-0.144	r=0.624**
Lower A-NiTi/ER SM	r=0.301**	r=-0.144		r=0.225
Lower A-NiTi/ER LB	r=0.002	r=0.624**	r=0.225	

[Table/Fig-10]: Correlation of SM and LB numbers between upper and lower archwires of A-NiTi/ER.
*p<0.05; **p<0.01; ***p<0.001; r, Pearson correlation coefficient number; 0.30<r<0.50 low positive correlation; 0.50<r<0.70, moderate positive correlation [17]; A-NiTi/ER: After NiTi/ER application

A-NiTi/ER, thereby, increasing plaque involvement [13]. Therefore, in present study, elastic ligature application was preferred instead of stainless-steel ligature wire. Despite this, it was observed that A-NiTi/ER was peeled after 4 weeks of intraoral usage. The peeling was more frequent between brackets in labial regions than posterior. The abrasive effect of tooth brushing and the contact between the coating material and the brackets can be considered as the reasons for coating loss [7,14,18]. Similarly, after 3 weeks of intraoral application, between 25-72% peeling of coated metallic archwires and colour stability loss were previously reported [8,13,18].

In the present study, the mean value of SM colony numbers in saliva was significantly lower before archwire application than A-NiTi and A-NiTi/ER application. The 1-week washout period, during which, all the orthodontic attachments, except brackets and molar tubes, were removed, could be the reason for this reduction [1]. Similar to the study results of Beyth N et al., in present study, SM colony numbers increased in saliva after applying both A-NiTi and A-NiTi/ER arches [19]. However, the difference in the type of surface characteristics does not statistically affect the mean value of SM colony numbers in saliva.

In saliva samples of the present study, statistically similar mean LB colony numbers were obtained before archwire application, after A-NiTi and A-NiTi/ER application, although it was lower after wash out period. This bacterium has a high retention capacity to the retentive areas that cannot be washed by saliva [20]. Consequently, the amount of LB in the oral flora increases after undergoing fixed orthodontic treatment [2]. Furthermore, it has been reported that patients with fixed orthodontic treatment do not achieve a significant decrease in LB level, despite an effective oral care implementation [21]. In our study, individuals had been under orthodontic therapy for more than 12 months, during which resident oral microflora developed. Hence, increased mean LB colony numbers during the study were expected. Decreased but statistically insignificant mean LB colony numbers before archwire application might suggest that the 1 week washout period could be insufficient to reduce these bacterial counts significantly.

The mean SM colony numbers on the A-NiTi and A-NiTi/ER were statistically similar. The mean LB colony numbers were statistically higher for A-NiTi/ER than A-NiTi. The reason for this result could be severe deterioration, greater surface roughness, and increased retentive areas, due to loss of the surface coating [13]. Probably, retention areas on the peeled archwire may have provided a local LB increase, by acting as retentive areas in caries cavities. It can be assumed that the increasing LB count on the retentive areas of A-NiTi/ER, led to more acidic microbial dental plaque, which has a bactericidal effect on SM. In the presence of fermentable carbohydrates in accumulated plaque, aciduric microorganisms,

such as SM, generate bacterial fermentation and lower the pH, by secreting acid. Such an environment will favor acid-tolerant bacteria. An ecological shift in the plaque microflora and a resultant increase in aciduric species, like SM and LB, can be induced [22,23]. LB can survive and reproduce at low pH. These bacteria produce acids at pH 4.5 and below. This low acidity is only observed in regions, where the dental plaque is dense. SM lowers the pH by secreting acid, providing an increase in LB colony numbers. Reproduction of SM results in a decrease in its colony numbers when the number of LB increases [24,25].

In accordance with our findings, and a higher bacterial adhesion on NiTi/ER wires in in vitro conditions has been presented in the literature [26,27]. In contrast, higher SM adhesion on uncoated NiTi wires and lower on coated NiTi, owing to the higher surface energy of uncoated NiTi archwires in vitro were indicated [12]. Raji SH et al., reported that A-NiTi/ER, exhibit lower surface roughness than uncoated NiTi wires and, consequently bacterial adhesion is reduced compared to uncoated NiTi wires [4]. *In vitro* studies that do not reflect clinical routine and in vivo studies performed with an inadequate number of individuals could account for these conflicting results.

In this study, dental archwires with the same surface characteristics were applied to both lower and upper dental arches (A-NiTi or A-NiTi/ER). According to results obtained from present study that there were no statistically significant differences for arch*archwire interaction in A-NiTi or A-NiTi/ER. This finding indicated that the amount of bacterial adhesion on upper and lower A-NiTi is statistically similar, and the amount of bacterial adhesion on upper and lower A-NiTi/ER are statistically similar, regarding SM and LB colony numbers. Similar SM and LB adhesion to the archwires of upper and lower dental arches, for both groups assessed in this study, may indicate the presence of homogeneous bacteria distribution in the oral microflora. This statement was confirmed by Pearson's correlation result, which showed the increase in the amount of SM and LB colony numbers on upper arches correlated with the increases on the lower arches for both A-NiTi and A-NiTi/ER [Table/Fig-10]. Moreover, this finding concurs with the literature [4,28].

According to Pearson's correlation coefficient results no statistically significant correlations were present between the wire and saliva samples for SM numbers. For LB colony numbers, a moderate positive correlation was found between saliva sample-upper archwire, and saliva sample-lower archwire, for both A-NiTi and A-NiTi/ER. The increased colony numbers of LB in saliva and on both archwires were related to each other. Correlation of LB numbers in both saliva and archwires can be explained by the high retention capacity of LB [20], and the retention areas generated by the archwires for LB adhesion.

According to this study results, the null hypothesis was rejected. Orthodontic archwires are coated to obtain aesthetic appearance. However, if this archwire locally increases the number of microorganisms causing white spot lesions during fixed orthodontic treatment, it may overshadow the achievement of an aesthetic smile. Clinically, it may be advisable not to apply NiTi/ER wires in patients with high caries risk.

LIMITATION

Applying coated arch wires to the metal orthodontic brackets instead of aesthetic brackets could be a limitation of this study. Because coating technology improves not only aesthetic characteristics of the wire but also mechanical properties as well. Another limitation of present study was that the 1-week washout period performed at the beginning of our study was not repeated before the application of second arch wire that exhibited different surface properties. In the future studies, using aesthetic brackets, and applying a washout period between the sequential applications of two different archwires, which were the limitations of this study, may be suggested. Additionally, the changes in SM and LB colony numbers might be explained in detail, with the assessment of changes in salivary pH value and salivary flow rate.

CONCLUSION

In conclusion, the amount of SM in saliva increased after the application of both archwires. Increased LB colony numbers were obtained on A-NiTi/ER than A-NiTi, after sequential intraoral usage of 4 weeks, in the same patients. The increase in LB colony numbers was moderately positively correlated with each other in saliva and A-NiTi and, similarly A-NiTi/ER. A low positive correlation was found between upper A-NiTi/ER and lower A-NiTi/ER, regarding SM colony numbers; moderately positive correlation was detected between LB colony numbers of upper and lower A-NiTi.

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