Comparative analysis of microorganism adhesion on coated, partially coated, and uncoated orthodontic archwires: A prospective clinical study

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Introduction: This study aimed to compare the microorganism adhesion on coated, partially coated, and uncoated orthodontic archwires after clinical use. The correlation between surface roughness (SR) and bacterial colonization was also evaluated. **Methods:** A total of 48 archwire segments (0.016 × 0.022-in) were equally divided into 4 groups: nickel-titanium coated, nickel-titanium partially coated, uncoated stainless steel, and uncoated nickel-titanium. The archwires were randomly inserted in a split-mouth study design. After 4 weeks of clinical use, the total number of microorganisms adhering to the archwire was quantified and transformed into colony-forming units. SR was evaluated using a profilometer. A one-way and two-way ANOVA, post-hoc Tukey test, paired-samples *t* test, and Pearson correlation coefficient were used for statistical analysis. **Results:** All the archwires presented microorganism adhesion, with the nickel-titanium–coated group demonstrating the highest value ($P < 0.001$). A statistically significant increase in SR was observed after clinical use for all groups ($P < 0.05$). No correlation between SR and bacterial adhesion was detected. **Conclusions:** Microorganism adhesion occurred on all of the archwires tested, especially on the esthetic fully coated. Because SR was not correlated with microorganism adhesion, future studies should evaluate the effect of surface free energy and superficial chemical changes on in-vivo microorganism adhesion. (Am J Orthod Dentofacial Orthop 2019;156:611-6)

Much progress has been achieved in orthodontics over the last few decades, particularly in terms of the quest to facilitate mechanics, obtain more precise diagnosis and treatment, and meet the esthetic demands of patients.[1] Among the esthetic appliances available on the market, there are lingual appliances, plastics aligners, and esthetic brackets, which are sold in the form of both conventional and self-ligating accessories. Esthetic brackets have become widely used, and esthetic archwires can be used to make these appliances minimally perceptible in the patient's appearance.

The esthetic archwires that are currently available on the market comprise metal archwires coated with esthetic materials such as Teflon, epoxy resin, and rhodium.[2] Initially, all surfaces of archwires were coated; however, they are also sold with coating only on the buccal surface because of the implications of coating on sliding mechanics.[3]

Coated metal archwires improve the appearance of fixed orthodontic appliances[4,5]; however, greater surface roughness (SR),[3,6-8] change of mechanical proprieties,[6,7,9] more corrosion,[8] problems related to color change,[10] and peeling of the coating have been observed.[3,6] In the literature, studies that have evaluated the mechanical and physical properties of different esthetic orthodontic archwires have been published.[3,6-10] However, the microbiologic aspects have scarcely been explored, and until recently, information regarding

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biofilm accumulation was limited to studies conducted in vitro.[2] Taha et al[11] performed a microbiologic evaluation of coated archwires removed from different patients and verified that adhesion in the in-vitro environment did not simulate the clinical situation in vivo.

Some authors have suggested that the increase in SR of archwires, their corrosion, and the debris occurring during their clinical use, could facilitate the adhesion of microorganisms.[12] However, there remains a scarcity of clinical evidence to confirm this correlation.[11,13]

Control of periodontal health and white spot lesions and prevention of caries are important factors for obtaining successful orthodontic treatment. Studies have shown that the use of orthodontic appliances promotes specific changes in the oral environment, including a reduction in pH and elevation of *Streptococcus mutans* levels in saliva and biofilm.[14-16] The adhesion of microorganisms to orthodontic devices may have an influence on enamel demineralization[17] and the development of periodontal diseases,[18] in addition to the possibility of interfering in orthodontic mechanics.[3,19,20] Therefore, studies that evaluate the microbiologic aspects of orthodontic materials should be encouraged.

Thus, this study aimed to compare the total number of microorganisms adhering to coated, partially coated, and uncoated orthodontic archwires after 4 weeks of clinical use. In addition, the correlation between SR and bacterial colonization was evaluated.

MATERIAL AND METHODS

This clinical study was approved by the local Research Ethics Committee (#1.384.568), and written informed consent was obtained from all participants.

The sample size calculation was based on data from a pilot study, and the number of microorganisms was used as a reference variable. The parameters used were a confidence level of 95%, a power of 80%, a standard deviation (SD) of 0.62, and a minimum difference of 1 between the means (PASS 11; NCSS, Kaysville, Utah). The minimum number of archwire segments was determined to be 10, and 20% was added to this value, thus resulting in 12 archwires segments.

Subjects aged between 18 and 30 years were enrolled in this study. The following inclusion criteria were established: presence of all permanent teeth up to first molars erupted, absence of caries lesions or fractures on the buccal surface of teeth, and absence of periodontal disease.

The following exclusion criteria were adopted: pregnancy, diabetes, absence of vertical space for bonding the orthodontic brackets to the mandibular teeth, and use of antimicrobial solutions or antibiotics in the last 3 months.

After prophylaxis with a rubber cup and pumice stone, the participants had stainless steel brackets (Straight wire, 0.022 × 0.030-in slot in McLaughlin, Bennett, Trevisi prescription; Kirium, Abzil/3M Unitek, São José do Rio Preto, Brazil) passively bonded to their first premolars, second premolars, and first molars on both the maxillary and mandibular arches in the 4 quadrants. The brackets were positioned with the slots aligned, using a rectangular steel archwire (0.019 × 0.025-in) as a guide, in a manner that would not cause tooth movement.[21] A single operator specializing in orthodontics and who was previously calibrated performed the bonding procedures.

Four types of rectangular orthodontic archwires (0.016 × 0.022-in) that were available on the market at the time of the present study were selected (n = 12 for each group): nickel-titanium completely coated with rhodium (group C; Esthetic Rhodium Super Elastic, Orthometric, Haidian, Beijing, China); nickel-titanium partially coated with Teflon (group PC; Teflon, Rocky Mountain Orthodontics, Denver, Colo); uncoated stainless steel (group UCSS; Orthometric, Haidian, Beijing, China), and uncoated nickel-titanium (group UCNiTi; Flexy Super Elastic, Orthometric, Haidian, Beijing, China). The coated and partially coated wire segments were tied into the slot, and the uncoated wire segments were tied juxtaposed to the gingival base of the bracket wings using 0.010-in stainless steel ligatures, as described by Silva et al.[22] These archwires were inserted in a split-mouth study design so that all the archwires were simultaneously exposed to the same oral cavity and in both arches (Fig). Randomization was concealed by sequentially numbered, opaque, sealed envelopes containing the archwire type and hemi-arch. All archwires were sterilized in an autoclave before use.[13] An investigator who was not involved in the trial prepared the envelopes.

After the archwires were inserted, the participants individually received basic instructions on oral hygiene and care regarding the orthodontic appliance. All the participants received the same commercial brand of orthodontic toothbrush and fluoridated toothpaste. The participants were also instructed not to perform mouthwashing with any oral antiseptic during the period of the experiment to avoid changes in the oral microbiota. Before removal of the archwires, the participants were asked not to perform oral brushing for a minimum of 12 hours before the appointment.[23] After 4 weeks of clinical use, the orthodontic archwire segments were carefully removed using Mathieu sterile tweezers.

To evaluate the number of microorganisms adhering to the orthodontic archwires, an archwire length of

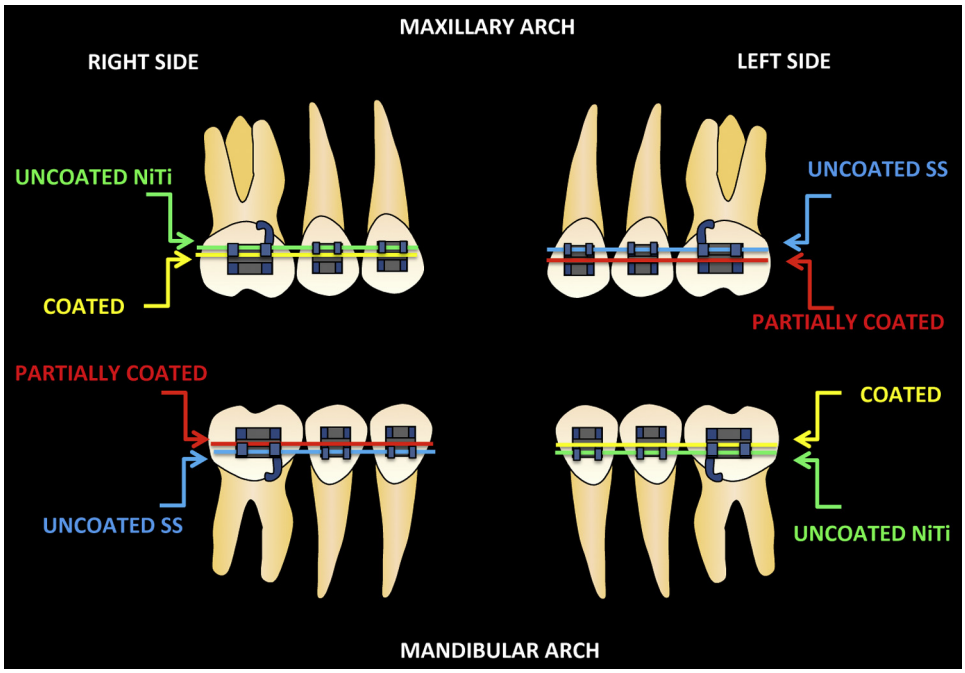

Fig. Schematic drawing of the archwires tested in the oral environment. The archwires were inserted in a split-mouth study design. The coated and partially coated wire segments were tied into the slot, and the uncoated wire segments were tied juxtaposed below to the gingival bracket wings.

7 mm was standardized, as counted from the most central part of the wire. After clinical use, the archwires were removed and individually placed in sterile Falcon-type tubes containing 10 mL of Brain Heart Infusion (Merck KGaA; Darmstadt, Germany). The adherent bacteria were then detached using a sonicator (CL-18; Fisher Scientific, Pittsburgh, Pa) for 3 pulses of 15 seconds (at a power of 50 W) and followed by 3 intermittent cooling periods of 15 seconds. After this, the tubes containing the microbial suspension were submitted to serial decimal dilution. An aliquot of each dilution was plated on blood agar plates to count the total microorganisms. The plates were incubated at 37°C in 5% CO_2 for 48 hours. After growth was verified, the microorganisms were submitted for counting, and the results were expressed in colony-forming units correlated with volume in milliliters and transformed into logarithmic scale ($\log_{10}$).

The roughness of the orthodontic archwires was measured before they were inserted in the oral cavity and after their removal using a profilometer (SJ-210; Mitutoyo Corporation, Tokyo, Japan).[24] Three consecutive readouts of 2.5 mm were taken on the buccal surfaces of the archwires, at a speed of 0.5 mm/s. The SR expressed in Ra (μm) was determined by the arithmetic mean of the 3 readouts in accordance with the ISO 1997 standard. A previously calibrated operator performed the SR evaluation.

Statistical analysis

To evaluate the reproducibility of the roughness measurements, 15 archwire segments were remeasured after 4 weeks. The paired-samples t test was conducted to determine the systematic error, and the Dahlberg formula ($Se^2 = \sum d^2/2n$) was used to determine the casual error.

A two-way ANOVA was applied to compare the coating characteristics (coated, partially coated, and uncoated) and time of evaluation (T0: archwire as-received, and T1: after clinical use). The one-way ANOVA and post-hoc Tukey tests were used to conduct a comparative analysis of microorganism adhesion and SR among the groups tested. The Student t test was used to compare the SR of the archwires before and after clinical use. The correlation between the final roughness and microorganism adhesion was tested using the Pearson correlation coefficient (r) with a 95% confidence interval (CI). The level of significance adopted was 5% ($P < 0.05$). SPSS statistical software (version 25; IBM, Chicago, Ill) was used for statistical analysis.

RESULTS

The reproducibility of the SR measurements was demonstrated by the fact that no significant systematic or causal errors were detected (paired-samples t test: $P = 0.12$ and Dahlberg = 0.2).

Table I. Results of 2-way ANOVA considering the coating characteristics and the time of evaluation

	DF	*SS*	*MS*	*F*	P *value*
Intercept	1	48.36000	48.36000	247.3342	0.000*
Time	1	8.45678	8.45678	43.2517	0.000*
Coating characteristics	2	6.86756	3.43378	17.5619	0.000*
Error	92	17.98829	0.19552		
Total	95	33.31264			

DF, degree of freedom; *SS*, sum of squares; *MS*, mean square.
*$P \leq 0.05$ (statistically significant).

Table II. Comparative analysis of microorganism count among the groups after clinical use

Groups	*Mean**,†	*SD*
C	11.80[a]	0.82
PC	7.01[b]	0.79
UCSS	8.15[bc]	1.37
UCNiTi	9.28[c]	2.13

Note. Bacteria counting was expressed on a logarithmic scale ($\log_{10}$).
*Indicates statistically significant differences ($P \leq 0.05$) as determined by ANOVA; †Different lowercase letters indicate statistically significant differences ($P < 0.05$) among the types of orthodontic wire, found by the Tukey test.

Patients who lost any archwire segment or bracket were eliminated from further analysis. Initially, 10 participants had 80 archwire segments inserted; however, only 6 participants maintained the devices in all the quadrants for 4 weeks. Therefore, the final sample comprised a total of 12 archwire segments per group.

There were significant differences in the coating characteristics of the wires, time of evaluation, and interactions among them (Table I). All the archwires demonstrated microorganism adhesion, with group C showing the highest bacterial biofilm count compared with the other groups (C: 11.8 [0.82]; UCNiTi: 9.28 [2.13]; UCSS: 8.15 [1.37], and PC 7.01 [0.79]; Table II).

Before clinical use, group C presented a statistically higher mean roughness than group UCNiTi ($P < 0.05$; 0.43 [0.21] vs 0.14 [0.13]; Table III), whereas group PC (0.28 [0.35]) and group UCSS (0.29 [0.18]) showed intermediate values with no statistically significant differences compared with other groups (Table III). After oral exposure, it was detected that group PC presented statistically higher roughness than all other types of archwires ($P < 0.001$; PC: 1.79 [0.41] vs C: 0.90 [0.37], UCSS: 0.59 [0.39], and UCNiTi: 0.57 [0.36]; Table III). In the intragroup evaluations, a statistically significant increase in SR was observed after clinical use for all groups (Table III). SR was not correlated with microorganism adhesion (Table IV).

Table III. Comparative analysis of roughness among groups and time (before and after clinical use)

	Groups				
	C	*PC*	*UCSS*	*UCNiTi*	P *value*
T0	0.43 (0.21)[a]	0.28 (0.35)[ab]	0.29 (0.18)[ab]	0.14 (0.13)[b]	0.034*
T1	0.90 (0.37)[a]	1.79 (0.51)[b]	0.59 (0.39)[a]	0.57 (0.36)[a]	<0.001*
P value	0.002†	<0.001†	0.028†	0.003†	

Notes. Values are mean ± standard deviation; the roughness values were expressed as the arithmetic mean roughness value (Ra = μm); and different lowercase letters in the horizontal direction indicate statistically significant differences ($P < 0.05$) among the groups, found by the Tukey test.
*Indicates intergroup comparison as determined by ANOVA; †Indicates intragroup comparison as determined by *t* test before and after clinical use for 4 weeks.

Table IV. Correlation analysis between roughness and microorganism adhesion

Groups	*R*	*95% CI*	P *value*
C	0.10	-0.64 to 0.75	0.75
PC	0.13	-0.48 to 0.66	0.68
UCSS	0.36	-0.45 to 0.85	0.24
UCNiTi	0.24	-0.54 to 0.80	0.43

DISCUSSION

Bacterial accumulation on orthodontic devices plays an important role in the pathogenesis of enamel demineralization during orthodontic treatment.[17] Orthodontic archwires are manufactured from different metal alloys, present different mechanical properties and SR values, and may also be coated with specific materials to improve their esthetic appearance during treatment. The scarcity of controlled clinical studies that compare the number of microorganisms adhering to different types of orthodontic archwires exposed to the oral cavity justifies the relevance of the present study.

Archwires of the same cross-section (0.016 × 0.022-in) were used to standardize the size and shape, allowing greater precision in the microbiologic evaluation. Moreover, this study was designed to enable all the archwires to be simultaneously exposed to the same factors, thereby eliminating biases such as differences in pH, food, cleaning, temperature, and bacterial flora, with each patient serving as his or her own control as well.[21]

The detection of total microorganisms was positive on all archwires tested, corroborating the findings of previous studies.[2,11,13] Our results showed a lower value of microorganism count in group PC (Table II), as was observed by Taha et al.[11] This finding may suggest the use of esthetic archwires with only the buccal

surface coated, especially in patients with greater cariogenic potential or periodontal problems.

A statistically significant increase in SR was observed on all archwires tested (Table III), which is consistent with the results of previous studies evaluating the intra-oral aging of nickel-titanium,[25] stainless steel,[26] and esthetic archwires.[6,27] The increase in SR was probably related to abrasion during toothbrushing, eating, and interactions between the archwires, brackets, and ligatures; this may be more evident in the groups with coated wires. Similar to the findings of Rongo et al,[3] group PC, followed by group C, had the highest SR values after 4 weeks of exposure to the oral cavity (Table III). The increased roughness observed in group PC may reflect differences with the type of coating materials investigated (ie, metal and Teflon), as different materials have distinct physical and chemical properties, including surface degradability.

Similar to the present study, previous in-vitro studies have shown a lack of correlation between SR of orthodontic archwires and microorganism accumulation.[2,11,16,17] However, Taha et al[11] reported a positive correlation between these factors in vivo, which was not consistent with the in-vitro data obtained in the same study. Abraham et al[13] also observed a correlation between the adhesion of *S. mutans*, SR, and surface free energy when comparing NiTi and copper-NiTi archwires. The results obtained in vivo by Taha et al[11] and Abraham et al[13] differed from those obtained in our study. However, the differences in strains, types of archwires tested, exposure to the oral environment, and conditions of the experiments may justify the conflicting results. The archwires evaluated in the previous studies[11,13] were used in different patients. However, in our study, all the types of archwires tested were exposed simultaneously to the same oral environment and were present in both the maxillary and mandibular arches of the same patient, thereby eliminating several biases. The different types of SR parameters should also be considered when analyzing the results.[6]

The absence of a correlation between SR and the number of microorganisms adhering to the archwire may be related to surface free energy[2,17] and superficial chemical changes.[25,28] Chemical changes occurring on the surface of archwires exposed to the oral cavity may influence bacterial adhesion, given that chemical elements expelled during the process of surface corrosion of archwires could act as antibacterial agents. Furthermore, polar interactions are an important mechanism in the initial stage of bacterial adhesion.[29] This result also may be due to the different materials investigated (rhodium, polytetrafluoroethylene, stainless steel, and nickel-titanium), as the initial adhesion of bacteria to surfaces also depends on the composition of the solid substratum.[30]

The information regarding the number of microorganisms on an archwire that would promote enamel demineralization is inconclusive, and studies on this topic must be encouraged. The development of oral diseases depends on various factors that act concomitantly, such as host susceptibility, substrate availability (eg, dental structure), dietary habits, type of bacteria, time, and several other factors.[31] However, it is known that to have 10^5 colony-forming units per milliliters of cariogenic bacteria in the saliva and oral plaque is considered a high risk for caries development.[32] This present investigation showed high bacteria counts for all archwire groups (Table II). At this time, we cannot infer that the number of bacteria detected would help predict future caries and lesion formation, but any device in an oral environment that accumulates biofilm in susceptible hosts requires attention and special care; especially for orthodontic patients.

Furthermore, this study intends to offer a database regarding accumulation of bacteria on different archwires types, offering to the clinician the best option based on host susceptibility. Consistent with our findings, previous studies[11,14-16] have demonstrated that brackets, adhesives, ligatures, and orthodontic archwires increase the concentration of microorganisms in the mouth. Because possible enamel demineralization around orthodontic brackets is 1 of the most common side effects during orthodontic treatment with a fixed appliance, preventing such lesions should be an important concern for orthodontists.[17]

The results obtained reinforced that strict attention must be paid to oral hygiene during the use of orthodontic appliances to avoid enamel demineralization and periodontal diseases. The clinician must be able to choose the best option regarding the patient profile, taking into account factors including the ability of plaque disorganization, tooth brushing frequency, dietary habits and caries, and periodontal disease risk evaluation.

CONCLUSIONS

1. A significant quantity of microorganisms was observed on all archwires after clinical use.
2. The esthetic fully coated archwire accumulated the most microorganisms.
3. The SR of all orthodontic archwires increased after 4 weeks of exposure to the oral environment.
4. SR was not correlated with microorganism adhesion.

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