

***In vitro* and *in vivo* biofilm adhesion to esthetic coated arch wires and its correlation with surface roughness**

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ABSTRACT

Objective: To evaluate the *in vitro* ability of esthetic coated rectangular arch wires to retain oral biofilms and *in vivo* biofilm formation on these wires after 4 and 8 weeks of clinical use and to correlate the findings with the surface roughness of these wires.

Materials and Methods: Three brands of esthetic coated nickel-titanium (NiTi) arch wires were selected. Arch wires retrieved after 4 and 8 weeks of intraoral use were obtained from 30 orthodontic patients. Surface roughness (SR) was assessed with an atomic force microscope. *In vitro* adhesion assays were performed using *Streptococcus mutans* (MS), *Staphylococcus aureus*, and *Candida albicans*. The amount of bacterial adhesion was quantified using the colony-count method. Paired *t*-test, analysis of variance, post hoc Tukey's test, and Pearson's correlation coefficient test were used for statistical analysis at the .05 level of significance.

Results: *In vitro* bacterial adhesion showed significant differences between wires in terms of MS adhesion ($P = .01$). All wires showed significant increases in SR ($P = .001$ after 4 weeks and .007 after 8 weeks) and biofilm adhesion ($P = .0001$ after 4 weeks and .045 after 8 weeks) after intraoral exposure. A significant positive correlation ($P = .001$ after 4 weeks and .05 after 8 weeks) was observed between these two variables *in vivo*, but the correlation was not significant for *in vitro* bacterial adhesion.

Conclusions: SR and biofilm adhesion increased after intraoral use at all time intervals. There was a positive correlation between SR and biofilm adhesion *in vivo* only. (*Angle Orthod.* 2016;86:285–291.)

KEY WORDS: Surface roughness; Esthetic wires; Biofilm adhesion

INTRODUCTION

The great demand for better esthetics during orthodontic treatment has led manufacturers to develop appliances that combine both acceptable esthetics for the patient and adequate technical performance for the clinician.¹ Although esthetic brackets made of ceramic or composite have brought a dramatic improvement in the appearance of the appliances,² metallic arch wires are still visible. Coated metallic and

fiber-reinforced arch wires have been introduced to complement esthetic brackets in orthodontics. Fiber-reinforced wires are still experimental and are not clinically popular. Stainless-steel or nickel-titanium (NiTi) arch wires are coated with polytetrafluoroethylene or epoxy resin.^{3,4} This coating improves the esthetics but creates a modified surface that can adversely affect friction, corrosion behavior, mechanical durability, biocompatibility, and plaque accumulation.⁵ These factors play an important role and can critically modify the efficiency of the orthodontic outcome.^{5,6} There are conflicting results from previous research concerning esthetic coated arch wires. An evaluation of sliding properties⁷ reveals that the plastic coating decreased the friction between arch wires and brackets. It has also been noted that the coating protected the underlying wire from corrosion. However, some authors have experienced changes in the color⁸ and coating split during usage in the mouth, thereby exposing the underlying metal.^{5,9,10}

Moreover, an investigation of surface roughness (SR) found that, both peeled and remaining coated

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Table 1. Characteristics of Arch Wires Used in the Study

Group	Manufacturer	Cross Section Size, inches	Composition	Coating Surface
Type 1	Ortho Organizers (Sao Marcos, Calif)	0.016 × 0.022	NiTi	All surfaces
Type 2	Forestadent (Pforzheim, Germany)	0.016 × 0.022	NiTi	All surfaces
Type 3	TP Orthodontics (Laporte, Ind)	0.016 × 0.022	NiTi	Labial surface

NiTi: indicates nickel-titanium.

areas showed a greater SR after oral exposure.¹¹ Increased SR can increase the coefficient of friction, which is an essential factor in determining the effectiveness of sliding tooth movement.⁶ In addition, rough areas create new locations for plaque retention, with impaired mechanical removal.¹²

Biofilm formation causes periodontal diseases¹³ and enamel decalcification.^{14,15} The periodontal side effects, such as pocket formation and bleeding on probing,¹⁶ are considered to be transient.¹⁷ In contrast, signs of enamel decalcification, such as white spot lesions, are frequently permanent.¹⁸ Although research have focused on the color, coating stability, mechanical properties, and surface characteristics of esthetic coated arch wires, there is a scarcity of information about SR and its effect on biofilm formation after a long duration of oral exposure. Therefore, the aim of this study was to evaluate the in vitro ability of esthetic coated rectangular arch wires to retain oral biofilms and in vivo biofilm formation on these wires after 4 and 8 weeks of clinical use and to correlate the findings with the SR of these wires.

MATERIALS AND METHODS

Three brands of esthetic coated rectangular arch wires were used for this study (Table 1), which included in vitro and in vivo parts.

Sample size calculation showed that in order to detect 0.05 μ m of difference in SR with a power of 0.80 and an alpha value of .05, 30 ("as received"; 10 of each kind) and 60 retrieved wire pieces (30 after 4 weeks and 30 after 8 weeks) would be required for this study.

In Vitro Part

For each brand, five pieces of 20-mm length were cut from the end of "as received" arch wires and sterilized under ultraviolet light.¹⁹

Bacterial Attachment on Orthodontic Wire

Quantitative detection of biofilm formation was done by viable bacterial cell counting.²⁰ *Streptococcus mutans* (MS), *Staphylococcus aureus* (SA), and *Candida albicans* (CA) were cultured individually in an Eppendorf tube containing 1.5 mL of brain heart infusion (BHI) broth soaked with 2-cm wire. After 40-hour

incubation at 37°C under aerobic conditions, each wire was washed twice in sterile phosphate-buffered saline (PBS; pH 7.2) and moved to another sterile Eppendorf tube.

For viable cell counting, each wire was sonicated in 1 mL PBS. The PBS was serially diluted to 1/10,000, and each 100 mL was spread onto a BHI agar plate. After incubation for 2–3 days, bacterial colonies were counted from each plate, and the relative colony-forming units were calculated.

Quantitative Biofilm Measurement

Wires were washed carefully with PBS to remove nonadherent bacteria. Then wires were stained with 0.5% crystal violet solution for 30 minutes, washed five times with distilled water, and left to dry at room temperature for 30 minutes. The crystal violet was then solubilized by the addition of 95% ethanol (200 μ L). The absorbance was determined at 595 nm using an enzyme-linked immunosorbent assay (ELISA) reader (TC 98 ELISA STRIP READER, TECO Diagnostics, Anaheim, Calif). The OD595 threshold value over which strains were considered to be significant biofilm formers was 0.5.²¹

In Vivo Clinical Part

This study was approved by the Ethics Committee of Mansoura University. An informed consent form was signed by every patient/parent. Retrieved arch wires were obtained from 30 orthodontic patients (18 females and 12 males) with a mean age of 16.37 ± 3.36 years. Fifteen patients (five for each kind of wire) were in the 4-week group, and the other 15 patients were in the 8-week group. All the patients were selected from the Orthodontic Department (Faculty of Dentistry, Mansoura University, Egypt). Patients displayed permanent dentition, had good oral hygiene and healthy gums look, with no signs of redness, edema, or bleeding during brushing, were motivated in terms of sustaining good oral hygiene, and were supplied with a standardized tooth brush, toothpaste, and dental floss. They did not use any antibiotics or antibacterial mouthwashes or undergo professional tooth cleaning during the period of the study.

All patients had straight wire, 0.022 × 0.030-inch brackets of the Roth system (Equilibrium 2, Dentaaurum).

Table 2. In Vitro Adhesion of *Streptococcus mutans*, *Staphylococcus aureus*, and *Candida albicans* to the Three Types of Wires

Wire	<i>Streptococcus mutans</i>		<i>Staphylococcus aureus</i>		<i>Candida albicans</i>	
	Mean	SD	Mean	SD	Mean	SD
Type 1	0.1208	0.0115	0.1258	0.0270	0.1221	0.0260
Type 2	0.1191	0.0303	0.0908	0.0269	0.0897	0.0179
Type 3	0.0928 ^{ab}	0.0182	0.1227	0.0255	0.1325	0.0172
ANOVA <i>P</i> -value	0.01		0.01		0.0001	

SD: indicates standard deviation. *P*: probability test used: analysis of variance (ANOVA) followed by post hoc Tukey's test.

^a Significant with wire 1.

^b Significant with wire 2.

Arch wires were ligated using 0.010-inch stainless-steel ligatures. After 4 or 8 weeks, the arch wires were removed carefully to avoid iatrogenic biofilm dislodgement. They were rinsed with an air/water spray to loosen the debris and were then air-dried. Each arch wire was placed into a self-closing sterilizing plastic bag. On the outer surface of each bag the name of the patient, the date of insertion and removal, and the type of arch wire were recorded. The bags were stored until the time of investigation. Straight pieces of 20-mm length were cut from the distal end of the arch wires using wire cutters.

Quantification of biofilm formation on the wires was done on five pieces of each type on the same day of removal as for the in vitro study.

Surface Roughness

SR of five pieces of "as received" and retrieved samples from each kind of wire after 4 and 8 weeks was analyzed using an atomic force microscope (AFM; AUTOPROBE CP-Research, model AP-0100, THERMOMICROSCOPES, Sunnyvale, Calif). The AFM was used with the following specifications: scan rate, 1 Hz; resolution: 256 × 256 line; used probe: Contact ultralevers used Proscan 1.8 software and IP 2.1 software was used for image processing. Three areas measuring 5 × 5 μm each, one in the center of the wire, one 2 mm left, and one 2 mm right of the labial surface of the wire segments, which were fixed on

a glass slide, were assessed. The average SR (R_a) of the remaining coating was evaluated.

Statistical Analysis

The data were examined for the normality of distribution with the Kolmogorov-Smirnov test. Data are expressed as mean value ± standard deviation (SD). Comparisons between two related groups were carried out by paired *t*-test, while for comparison between more than two groups, analysis of variance (ANOVA) followed by post hoc Tukey's test was used. The Pearson's correlation coefficient test was used to assess correlation between different variables. Differences were considered statistically significant when $P < .05$. SPSS for Windows (Version 17.0, SPSS, Chicago, Ill) was used.

RESULTS

In Vitro Results

In vitro adhesion of MS, SA, and CA to the wires is summarized in Table 2. For MS adhesion, type 3 showed the lowest mean value (0.0928 ± 0.0182), while type 2 showed the lowest mean value for SA and CA (0.0908 ± 0.0269 , 0.0897 ± 0.0179 , respectively). Tukey's test indicated that type 3 had significantly lower MS adhesion than the other two types. The three types of wires did not differ significantly with respect to SA and CA. However, ANOVA analysis showed

Table 3. Surface Roughness (R_a , μm) Mean and Standard Deviation (SD), Analysis of Variance (ANOVA) Analysis Results, and Tukey Post Hoc Comparison Between "As Received" and Retrieved Wire

Wire	Roughness, "As Received"		Roughness, 4 wk		Roughness, 8 wk		<i>P</i>
	Mean	SD	Mean	SD	Mean	SD	
Type 1	0.0656	0.0169	0.1746 ^a	0.0323	1.1000 ^{ab}	0.3125	0.0001
Type 2	0.1885 ^c	0.0403	1.4479 ^{ac}	0.3908	1.6106 ^{ac}	0.3875	0.0001
Type 3	0.1486 ^{cd}	0.0294	0.1723 ^d	0.0440	1.2923 ^{ab}	0.2939	0.0001
<i>P</i>	0.0001		0.0001		0.007		

P: probability test used: repeated ANOVA followed by post hoc Tukey's test.

^a Significant with "as received" group.

^b Significant with week 4 group.

^c Significant with wire 1.

^d Significant with wire 2.

Table 4. Biofilm Adhesion on Different Types of Wires at Different Time Intervals

Wire	Biofilm, 4 wk		Biofilm, 8 wk		P2
	Mean	SD	Mean	SD	
Type 1	0.5660	0.0770	0.8179	0.1979	0.003
Type 2	0.7854 ^a	0.2019	0.9547	0.2689	0.098
Type 3	0.4767 ^b	0.1092	0.6883 ^b	0.2044	0.01
P1	0.0001		0.045		

SD: indicates standard deviation. P: probability. P1: test used, analysis of variance (ANOVA) followed by post hoc Tukey's test. P2: Significance between 4 weeks and 8 weeks (test used, paired *t*-test).

^a Significant with wire 1.

^b Significant with wire 2.

a significant difference between the different wires for MS, SA, and CA ($P = .01$).

SR of "as received" wires is shown in Table 3. Types 1 and 2 showed the lowest and highest R_a values ($R_a = 0.0656 \pm 0.0169 \mu\text{m}$ and $0.1885 \pm 0.0403 \mu\text{m}$, respectively). There was a significant difference in SR among studded wires according to both ANOVA analysis ($P = .0001$) and Tukey's test.

The Pearson's correlation coefficient test showed a nonsignificant correlation between SR and bacterial adhesion (Figure 1).

In Vivo Results

Surface roughness. The SR parameters of "as received" and retrieved wires are shown in Table 3. In general, SR of the three types of wires increased at all the time intervals of oral use compared with the "as received" values. Type 2 showed the highest mean value of SR after 4 and 8 weeks of oral exposure (1.4479 ± 0.03908 and 1.6106 ± 0.3875 , respectively), whereas the lowest mean value was recorded for type 3 after 4 weeks (0.1723 ± 0.0440) and for type 1 after 8 weeks (1.1000 ± 0.3125). ANOVA analysis showed significant difference in SR between the three types of studded wires at all the time intervals ($P = .0001$ after 4 weeks and $P = .007$ after 8 weeks). The results of the Tukey's test revealed a significant difference between type 2 and the other two types after 4 weeks and between type 2 and type 1 after 8 weeks. Three-dimensional images of AFM of type 2 are seen in Figure 2.

Biofilm adhesion. A paired *t*-test was used for comparing biofilm adhesion after 4 and 8 weeks of oral use (Table 4). Biofilm adhesion increased significantly by increasing the time of oral use in type 1 ($P = .003$) and type 3 ($P = .01$). However, the increase was not significant in type 2 ($P = .098$). ANOVA analysis indicated a significant difference in biofilm adhesion among the three wire types ($P = .001$ after 4 weeks and $.045$ after 8 weeks). The Tukey's test

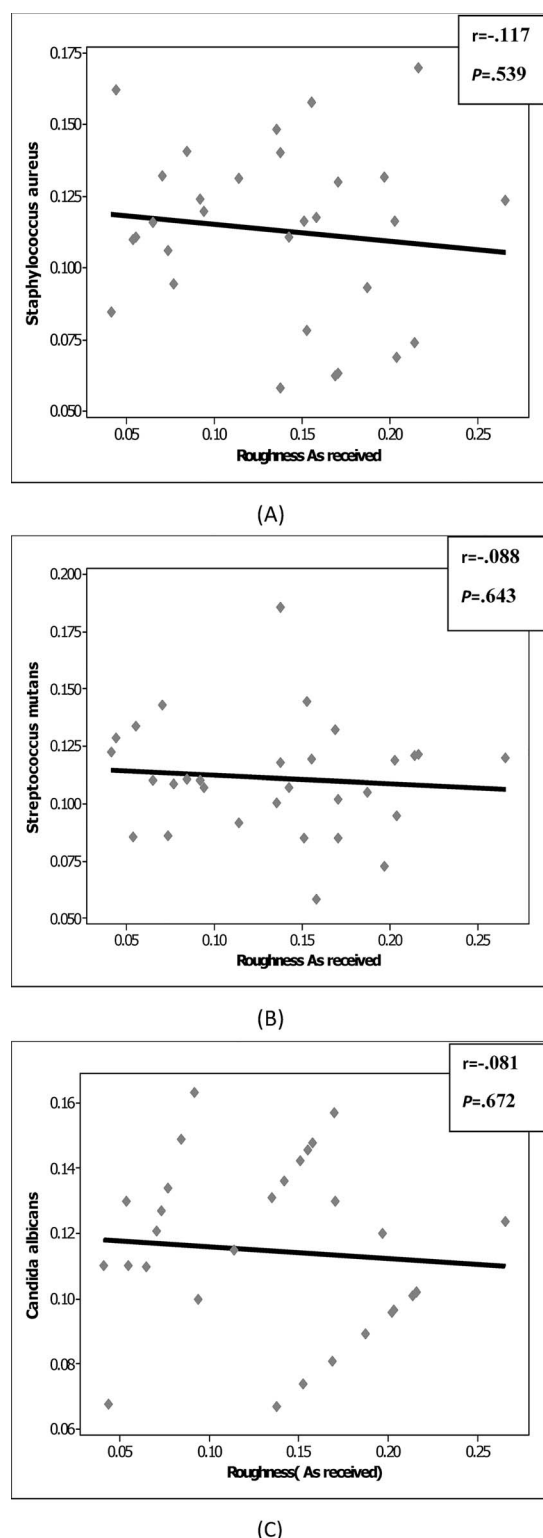
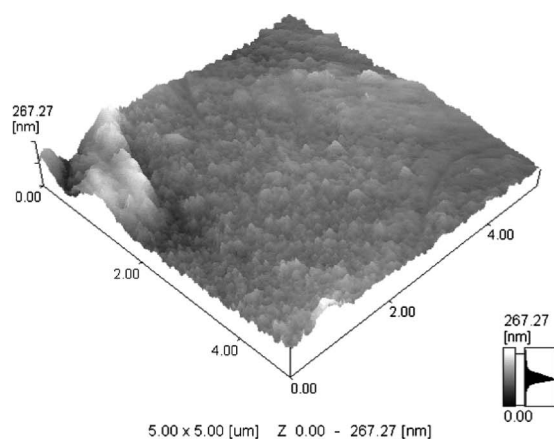
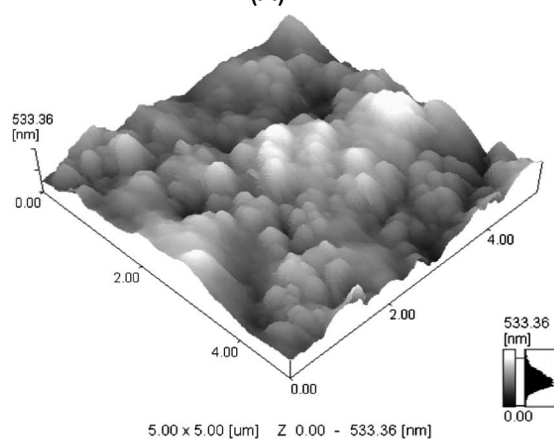


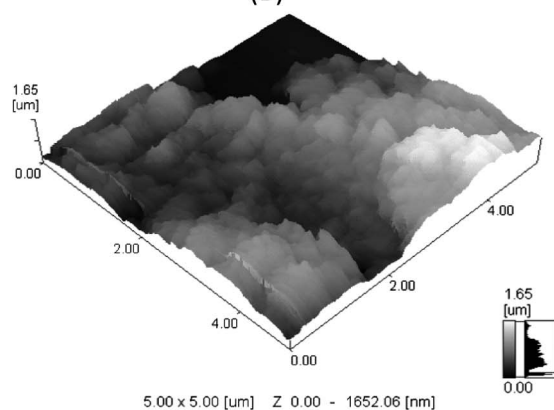
Figure 1. Pearson correlations between SR and biofilm adhesion to "as received" wires. (A) *Streptococcus mutans*; (B) *Staphylococcus aureus*; (C) *Candida albicans*. *r* indicates the Pearson correlation coefficient and *P*-values for the variables examined.



(A)



(B)

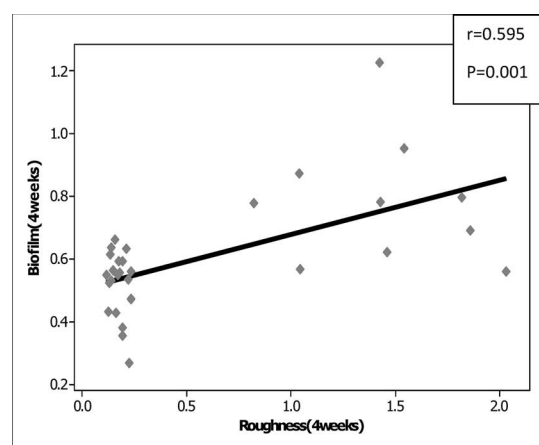


(C)

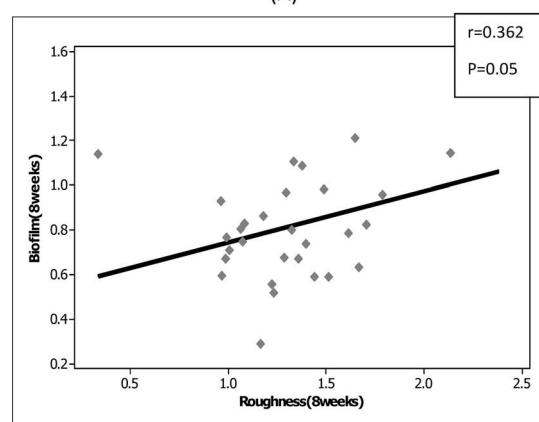
Figure 2. Three-dimensional atomic force microscope topography images ($5 \times 5 \mu\text{m}$) of the (A) “as received” specimens (B) after 4 weeks and (C) after 8 weeks of type 2 wire use.

revealed significantly high biofilm adhesion on type 2 compared with type 1 and type 3 after 4 weeks and on type 3 only after 8 weeks.

The Pearson's correlation coefficient test revealed a significant positive correlation between SR and



(A)



(B)

Figure 3. Pearson correlations between SR and biofilm adhesion to the retrieved wires (A) after 4 weeks and (B) after 8 weeks. r indicates the Pearson correlation coefficient and P -values for the variables examined.

biofilm adhesion (Figure 3). A greater degree of correlation was observed after 4 weeks of intraoral use ($r = 0.595$, $P = .001$).

DISCUSSION

Arch wires represent an ideal model for the study of material alterations occurring in vivo. They can be removed and studied during the regular patient treatment visits without implications for the advancement of treatment. Insertion of orthodontic wires creates new surfaces available for biofilm formation. Knowledge regarding the growth and adhesion of cariogenic bacteria to orthodontic materials will offer a better way of preventing white spot lesions. The scope of the present study was to evaluate in vitro and in vivo biofilm adhesion to esthetic wires.

In the present study, quantitative SR analysis performed by AFM showed significant differences among “as received” tested wires. This is likely attributable to the manufacturing technique and the

different effects of surface treatment. This hypothesis was confirmed by the fact that SR measured for various products from the same batch was quite homogeneous.^{5,6} In the in vitro part of this study, although type 2 was the roughest one, it did not produce significantly higher MS adhesion. Moreover, type 2 showed the least adhesion of SA and CA. This can be partially explained by the relatively minor differences in SR. Previous studies^{22,23} reported that minor variations in SR have no significant effect on bacterial adhesion. In addition, there are other factors, such as surface free energy and physicochemical properties, that affect the bacterial retaining capacity of dental materials.^{24–26} These results are confirmed by the correlation results (Figure 1). In agreement with our in vitro results, other studies^{20,27,28} have found no significant relationship between SR and bacterial adhesion to orthodontic materials.

After clinical use, there was a significant increase in SR parameters for all types of wires. This increase became more pronounced with an increase in the time of intraoral exposure from 4 to 8 weeks. This could be explained by the abrasive influence of tooth brushing, wearing from food, and the interaction between arch wire coating and bracket edges, which causes peeling of some coating, with the presence of areas of remaining coating and areas of metallic exposure increasing SR.^{4,11} These findings were in agreement with those of previous studies evaluating intraoral aging of NiTi,²⁹ stainless-steel,³⁰ and esthetic coated arch wires.^{4,11,31} Among the retrieved samples, type 2 was the roughest one and showed the greatest increase in SR after 4 weeks of oral exposure, but the increase was not significant after 8 weeks. This might be due to a considerable amount of coating delamination after 8 weeks.

In vivo biofilm detection was positive on all types of wires, and it increased with the duration of exposure. This could be explained by the large increase in SR after oral exposure. Another in vivo study²³ revealed that the threshold SR for bacterial adhesion is 0.2 μm . As shown in Table 3, SR values of all types of wires after oral exposure were greater than 0.2 μm . Rough surfaces provide opportunities for bacterial adhesion by increasing the surface area, providing suitable niches for bacteria and impairing bacterial colony dislodgment.^{9,10,32} Where the biofilm first develops within the valleys of uneven surfaces by irreversible attachment of planktonic pioneer bacteria, smoothing the rough regions.³³ In addition, changes in SR of greater than 0.1 μm influence the contact angle, thereby changing the surface free energy values, which comprise the second surface characteristic affecting bacterial adhesion to orthodontic materials.^{20,27,28,34} These results are confirmed by the correlation results, which revealed

a significant positive correlation between SR and in vivo biofilm adhesion (Figure 3).

Our in vivo results are inconsistent with our in vitro results and with previous in vitro results.^{20,26,27} These conflicting results indicate that the protocol for in vitro investigation cannot simulate the complex clinical situation. For this reason, retrieval analysis conducted on dental materials has recently received increasing interest. This type of analysis provides critical information concerning the performance of the materials in the environment in which they are intended to function.

The limitation of this study is that the periodontal parameters of pocket depth, bleeding on probing, and plaque index were not recorded at the time of insertion and removal of arch wires. In addition, the impossibility of standardized eating habits and the absence of a diary of the oral hygiene are also limiting factors.

CONCLUSIONS

- SR differs in the three types of “as received” wires.
- SR of retrieved esthetic coated arch wires increases after use in vivo.
- A significant amount of biofilm was found on all wire types after oral use.
- A positive correlation was found between the SR and biofilm adhesion in vivo, but no correlation was found in vitro.

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