ORIGINAL ARTICLE

The Influence of Gold, Rhodium, and Flexy Blue Titanium Esthetic Surface Coating of NiTi Archwire on Mutans Streptococci Adhesion: An In Vitro Study

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Abstract

Purpose: Orthodontic archwires play an important part in the enamel demineralization during orthodontic treatment. Dental caries is thought to be caused by the adhesion and colonization of mutans streptococci on these surfaces, followed by the formation of pathogenic plaque. This research was conducted with the purpose of testing and comparing the adhesion of mutans streptococci to a variety of aesthetic archwires as well as a conventional archwire made of NiTi.

Materials and Methods: Four types of nickel-titanium archwires with round cross-section 0.016 inch were used in the current study, one type uncoated NiTi and three types of coated NiTi (rhodium, gold, and flexy blue). After two hours of agitation in 2 ml of sterile Unstimulated Whole Saliva (UWS), 5 pieces of each archwire were incubated in a *streptococcus* mutans suspension for 5-, 90-, and 180-minutes time interval. Bacterial adhesion was assessed by a microbial culture technique and the amount of bacterial adhesion was counted by colony forming unit.

Results: There were no statistically significant differences in mutans streptococci adhesion among archwires at (90 and 180 minutes), while at 5 minutes, the mutans streptococci adhesion on gold-coated and rhodium-coated were significantly less than uncoated NiTi archwires.

Conclusion: Clinical use of esthetic-coated archwires may provide the same risks for bacterial adhesion compared with uncoated conventional archwires and increased mutans streptococci adhesion was significantly related to longer incubation time.

Keywords: Archwire Adhesion; Surface Coating; Mutans Streptococci; Esthetic Archwires; NiTi Alloys.

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1. Introduction

When an orthodontic appliance is in place, there is a greater potential for the plaque formation on additional surfaces, leading to an increase in the number of bacteria found in the oral cavity [1]. Plaque retention on orthodontic appliances used in treatment might lead to an increase in biofilm production during orthodontic treatment, which can cause gingival inflammation and demineralization of enamel, resulting in white spots and dental caries, because these lesions are unattractive, unhealthy, and possibly irreversible, and their prevention has been an important concern for orthodontists [2]. After bonding orthodontic brackets, increased amounts of mutans streptococci were discovered in the oral cavity, organic acids produced by mutans streptococci cause enamel demineralization, and S. mutans is the most important bacteria, due to its evident activity in forming insoluble extracellular polymer and acid production [3]. Surface properties of biomaterials, particularly surface roughness and surface free energy (SFE), influence in vitro bacterial adherence, which is important in providing a habitat for caries-causing microbes such as *Streptococcus* mutans [4]. Rougher surfaces may increase bacterial attachment more than SFE because they improve adhesion regions and hinder bacterial colony dislodgment [5]. Due to the high demand for greater esthetics during orthodontic treatment, manufacturers have created appliances that offer both patient-acceptable esthetic and clinician-acceptable technical performance [6]. Patients' concerns regarding esthetics are not just limited to malocclusion correction; they also extend to the appearance of orthodontic appliance. Due to the availability of esthetic materials, many people are currently seeking orthodontic treatment [7]. Most of the fixed orthodontic appliances are metallic and/or silver in color and are quite revealing to the outside environment. Manufacturing brackets from materials like ceramic or plastic can enhance their aesthetics. Alternatively, the archwires' aesthetic color can be produced by coating them with epoxy resin, Teflon, rhodium, or a combination of silver and biopolymers, as well as 24K gold [8]. Gold/rhodium-coated archwire that is used to increase shine and aesthetics compared to other coating materials (coated with resin epoxy and PTFE), it is more able to reduce the surface's porosity and roughness [7].

The Flexy Blue-NiTi archwire was treated by oxidation under high temperature to enhance properties and produce smooth and more homogeneous surface in comparison with the conventional NiTi archwire. Even though these coatings improve aesthetics, the surface they are applied to can be modified in a way that can have a negative impact on a variety of attributes, including corrosion behavior, mechanical durability, biomechanics, and plaque accumulation [9-11]. As a result, examining whether coated archwires provide greater risk than uncoated ones is essential. Previous studies concentrated on the surface features, color, coating stability, and mechanical properties of coated archwires [12, 13]. Few research has looked at the degrees of bacterial adherence to various types of orthodontic archwires to see which material has the greatest retention capacity for mutans streptococci. Previous studies mostly focused on the physical and mechanical features of fixed orthodontic device components. The current study was carried out to quantitatively assess and compare the effect of flexy blue titanium, gold, and rhodium coating of NiTi arch wires on mutans streptococci adhesion with respect to incubation time.

2. Materials and Methods

2.1. Experimental Group's Preparation

Four types of (NiTi) arch wire with round cross-section (0.016 inches) three types coated (Rhodium, Gold, and Flexyblue), and one type uncoated were used as shown in (Table 1).

Table 1. Investigated orthodontic archwires

Name of the arch wire	Coating	Manufacturer
Flexy® niti	Uncoated	Orthometric company Brazil
Flexyblue Ti®	Oxid layer	Orthometric company Brazil
Fantasia®	Rhodium	IOS company USA
Royal®	Gold	IOS company USA

Orthodontic archwires were extracted from the supplier's packaging and their distal (10mm) extremities were removed and discarded. Each type of

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archwire was then divided into five pieces that were (24+1) millimeters in length. The sample size that was advised for each subgroup was five wire pieces, totaling 45 pieces for each type of arch wire. All wire segments were sterilized in an autoclave at 121 degrees Celsius and 15 pounds per square inch for 20 minutes [14, 15].

2.2. Isolation of Mutans *Streptococci* (S Mutans)

Pure isolates of *mutans streptococci* from stimulated saliva were used in all in vitro tests. Seven patients between the ages of 14 and 18 years who appeared healthy volunteered for the current study. Each person was instructed to chew a piece of paraffin gum (0.5 gm) for one minute, expectorate to remove any remnants of food, and then chew the same piece of gum for another minute while saliva was collected in a sterile screw-top bottle [16].

Salivary samples were homogenized in a vortex mixer for one minute, and then ten-fold serial dilutions were made by adding 0.1 ml of the homogenized saliva to 0.9 ml of sterile phosphate buffer saline (PH=7). From the dilution (10^{-1}-10^{-5}), 0.1 ml of the saliva was then removed and spread on mitis salivarius bacitracin agar (MSBA) in duplicate by a sterile microbiological spreader. These plates were first incubated anaerobically with gas back at 37°C for 48 hours, followed by aerobic incubation for 24 hours at the same temperature [17, 18].

2.3. Unstimulated Saliva Collection

Saliva was collected from healthy individuals, who had to wait at least two hours before eating, drinking, or brushing their teeth in order to be suitable for the saliva collection. These people had no periodontal lesions or tooth caries at the time of saliva collection. To reduce the impact of diurnal variability in salivary content, saliva was collected between 7:00 and 8:00 AM. Spitting was used to collect the saliva in a sterile tube. To remove any cellular debris from the saliva sample, it was centrifuged at (3500RPM) for 10 minutes. Immediately following filter sterilizing through a cellulose acetate membrane filter 0.2µm, the resultant supernatants were used [19, 20]. Saliva was roughly assessed for pH using sensitive pH paper; saliva with pH readings outside the allowed range (6.5-7.2) was not included in the current study.

2.4. *Streptococcal* Adhesion to Orthodontic Archwires

Five pieces of each kind archwire were incubated for two hours in 2 ml of unstimulated whole saliva with agitation at 25 to 30 °C [19, 21]. The pieces were rinsed with phosphate-buffered saline solution 3 times. After that, each 5 pieces of arch wire were incubated at 37°C for different time intervals (5, 90, and 180) minutes with agitation in a 5 ml suspension of bacteria at 10^{7} -10^{8} /ml. To eliminate any non-adherent bacteria, the pieces of arch wires were then immediately rinsed with PBS two times [22].

2.5. Culture of Bacterial Adhesion

After being washed with phosphate-buffered saline for each experiment, the pieces of arch wire, along with any bacteria that were adhering to them in each tube, were treated with 2 ml of 0.25% trypsin/EDTA for 45 minutes at 37°C in an aerobic environment, in order to detach the adherent bacteria [22]. After that, 0.1ml from each tube was inoculated into selective media (MSB agar) plates and incubated anaerobically using a gas pack for 48 hours at 37°C. The tested sample during each time interval was carried out in triplicate, and then the average was calculated.

2.6. Counting Method

The plate counting method, also known as the spread plate method, was employed in the current study to count the number of cells that were actively growing and dividing in a sample. This approach depends on the bacteria's capacity to establish a colony on a nutritional medium that is visible to the naked eye [23] (Figure 1). The number of colonies on countable plates was counted (depending on the dilutions), and the colony-forming unit was calculated by multiplying that number by the dilution factor [24].

CFU/ml = No. of colony in plate × recorded dilution of the tube

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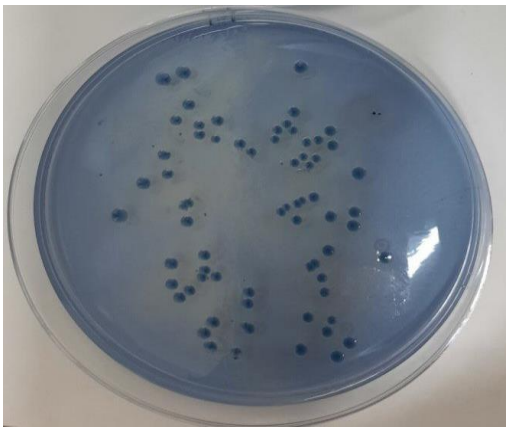

Figure 1. Bacterial colonies on the countable plate

2.7. Statistical Analysis

All the data of the samples were collected and statistically analyzed by using SPSS software version 26.

Statistical analyses were performed including median and standard deviation. Kruskall-Wallis H test, Mann-Whitney U test, and Pairwise comparison test were used for multiple comparisons. A value of $P<0.05$ was considered significant.

3. Results

Table 2 shows the number of *streptococcus* mutans adhesion in three times interval (5, 90, and 180) min for different types of archwire, which showed that the highest bacterial adhesion was in 180 min followed by 90 min and the lowest in 5 min. To compare the number of *streptococcus* mutans adhesion among three time intervals, the Kruskall-Wallis H test was used and showed significant differences with time intervals for all types of archwire. A pairwise comparison test was used to compare between each two time intervals and showed a significant difference between (5 and 180) min.

The result indicates that the incubation time effect on the amount of bacterial adhesion, S mutans adhesion was increased significantly by longer incubation time.

The descriptive statistics (Table 3) showed the number of *mutans streptococci* adhesion for different types of archwire in three time intervals (5, 90, and 180) min, which showed the highest bacterial adhesion for uncoated NiTi archwire in all incubation time, followed by flexyblue NiTi and the lowest for Rhodium and gold coated. Kruskall-Wallis H test was used for the comparison of bacterial adhesion among the four types of archwires, which demonstrated that there was a statistically significant difference between the different types of archwires at 5 minutes, but at 90 and 180 minutes, there was no statistically significant difference.

A pairwise comparison test for each pair of archwires types showed a substantial difference between uncoated and Rhodium-coated NiTi, as well as between uncoated and gold-coated NiTi (Table 4).

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Table 2. Statistical analysis of the number of adhering mutans streptococci at various time intervals for all types of archwire

Type of Archwire	Duration	Descriptive Statistic				Durations' Comparison				
						Kruskall-Wallis H-test		Pairwise comparison test		
		Median×10^3	SD×10^3	Max×10^3	Min×10^3	X^2	p-value	5-90	5-180	90-180
NiTi	5 min	3	2.64	7	2	7.2	0.027 (S)	0.18 (NS)	0.007 (S)	0.18 (NS)
	90 min	26	3.21	31	25					
	180 min	40	12.74	61	38					
Flexy Blue	5 min	1	0.57	2	1	7.2	0.027 (S)	0.178 (NS)	0.007 (S)	0.178 (NS)
	90 min	4	1	5	3					
	180 min	10	5.03	16	6					
Rhodium	5 min	1	0.57	1	0	6.7	0.034 (S)	0.85 (NS)	0.029 (S)	0.128 (NS)
	90 min	3	1.52	4	1					
	180 min	6	0.57	7	6					
Gold	5 min	1	0.57	1	0	6.5	0.038 (S)	0.134 (NS)	0.011 (S)	0.295 (NS)
	90 min	3	1.52	5	2					
	180 min	6	2	8	4					

Table 3. Statistical analysis of the number of adhering mutans streptococci on different types of archwire in each time interval

Duration	Group	Descriptive statistic				Groups' comparison (Kruskall-Wallis H test)	
		Median×10^3	SD×10^3	Max×10^3	Min×10^3	X^2	p-value
5 min	NiTi	3	2.64	7	2	7.8	0.048 (S)
	Flexyblue	1	0.57	2	1		
	Rhodium	1	0.57	1	0		
	Gold	1	0.57	1	0		
90 min	NiTi	26	3.21	31	25	7.1	0.068 (NS)
	Flexyblue	4	1	5	3		
	Rhodium	3	1.52	4	1		
	Gold	3	1.52	5	2		
180 min	NiTi	40	12.74	61	38	7.6	0.053 (NS)
	Flexyblue	10	5.03	16	6		
	Rhodium	6	0.57	7	6		
	Gold	6	2	8	4		

Duration	Group	Descriptive statistic				Groups' comparison (Kruskall-Wallis H test)	
		Median×10^3	SD×10^3	Max×10^3	Min×10^3	X^2	p-value
5 min	NiTi	3	2.64	7	2	7.8	0.048 (S)
	Flexyblue	1	0.57	2	1		
	Rhodium	1	0.57	1	0		
	Gold	1	0.57	1	0		
90 min	NiTi	26	3.21	31	25	7.1	0.068 (NS)
	Flexyblue	4	1	5	3		
	Rhodium	3	1.52	4	1		
	Gold	3	1.52	5	2		
180 min	NiTi	40	12.74	61	38	7.6	0.053 (NS)
	Flexyblue	10	5.03	16	6		
	Rhodium	6	0.57	7	6		
	Gold	6	2	8	4		

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Table 4. Comparisons between each two types of archwires

Duration	Groups	Pairwise comparison
5 min	(niti-flexyblue)	0.145
	(niti-rhodium)	0.015
	(niti-gold)	0.015
	(flexyblue-rhodium)	0.332
	(flexyblue-gold)	0.332
	(Rhodium-gold)	1.0

4. Discussion

The insertion of orthodontic appliances acts as a medium for plaque accumulation, raising the quantity of Streptococcus mutans bacteria in the oral cavity [25]. Dental caries and periodontal disorders are thought to be primarily caused by dental plaque and a biofilm. White spot lesions are a common unwanted side-effect of orthodontic treatment [26]. Orthodontic archwires are an essential component of fixed orthodontic equipment and provide a favorable habitat for oral microorganisms. One strategy for preventing enamel decalcification and periodontal diseases is to reduce the likelihood of oral microorganisms adhering to the surface of the archwires and subsequent growth around the fixed orthodontic appliances [27]. The materials of the orthodontic appliance that are employed will determine the level of adherence and growth potential of the bacteria [28].

The finding for this study showed there were no statistically significant differences in the adhesion rates of mutans streptococci between 4 types of wires at 60- and 90-min time intervals, but there were

statistically significant differences between gold and rhodium when compared with uncoated NiTi archwire at a 5-min time interval. The results show that there was a difference in the number of bacterial adhesions among the wires but the difference was not statistically significant which means the esthetic coating archwires had no effect on the in vitro adhesion of mutans streptococci. It seems that the difference between materials, including orthodontic wires in bacterial adhesion, appears to be attributable to differences in surface properties such as surface roughness and surface free energy [29, 30]. Lee, *et al.* [4] and Abraham, *et al.* [1] Reported a correlation between increased bacterial adhesion and increased surface roughness and surface free energy. They came to the conclusion that higher surface roughness led to higher bacterial adherence because it increased surface area and retention points. By contrasting the surface characteristics of coated and uncoated orthodontic wires, a number of studies have demonstrated that the coating may not have a significant influence on the surface features of the wires [31]. According to studies, ion implantation of rhodium and gold coating did not only fail to significantly reduce the surface roughness of NiTi wires, but instead increased it [32, 33]. It has been found that during the processing of gold-plated archwires, unequal cooling from high temperatures leads to internal structure rearrangements involving volume change that cause internal stress which consequently might generate cracks of the outer protective layer and accelerating corrosion, hence augmenting the surface roughness [34, 35]. A study by Asiry *et al.* [36] proved that rhodium-coated wires were categorized as medium roughness which was comparable with the conventional uncoated wires. Many surface treatments have been adopted for surface passivation and reduction of alloy corrosion, such as electro-polishing and oxidation in air at high temperatures. Regarding air oxidation at high temperatures, Flexyblue-NiTi archwire was treated by oxidation under high temperatures to enhance the properties that change the color of archwire to light blue. The oxide layer on the alloy's surface becomes thicker and increases with increasing oxidation temperature and time [37]. This feature may be the reason why this study did not find significant differences in the bacterial count across the various types of materials that were examined, which agrees with Oliveira, *et al.* [38] that found Biofilm accumulation risks associated with the clinical use of esthetic-coated orthodontic wires may be comparable to those associated with the use of uncoated archwires. The finding of this study disagree swith Kim *et al.* [39] found that covering orthodontic wires in gold and rhodium may lower the quantity of bacteria that adhere to the wires. This may be due to the low surface free energy of gold and rhodium in comparison to uncoated NiTi.

On the other hand, saliva is crucial to the mutans streptococci's ability to adhere to archwires. The presence of a salivary pellicle may help to explain this, which instantly covers the orthodontic wires, thereby causing the properties of the adsorbed salivary protein layer to govern, to a large extent, the microorganism adhesion to the surfaces of the archwires [40, 41]. The presence of histamines, lysozymes, and lactoperoxidase in saliva, all of which have remarkable antibacterial capabilities, may aid in reducing differences in bacterial adhesion among archwires. Saliva coating also decreases the surface energy of the materials [29, 42].

In terms of the influence of incubation time, extended incubation time increased the number of bacterial adhesion for all types of archwires and was maximum after 3 hours of incubation than 5 and 90 minutes, this could be in agreement with the findings of Ahn *et al.* [43, 44] who showed that higher adherence of mutans streptococci occurs as a result of prolonged incubation times.

5. Conclusion

There was no significant difference in mutans streptococci adherence among the tested archwires when colony counting was employed.

Coated esthetic archwires may pose comparable risks for biofilm accumulation compared to uncoated archwires.

A longer incubation time results in an increase in the number of mutans streptococci that adhere to the archwires.

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