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Assessment of Mutans Streptococci Adhesion on Different Esthetic Coatings of Nickel-Titanium (Niti) Archwires: An In Vitro Study

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Abstract

Plaque and enamel demineralization can occur when orthodontic archwires are present in the oral cavity because they provide a unique surface for bacterial adhesion. Bacterial biofilm plays a major role in the development of complications such dental caries and periodontal disease. The purpose of the study was to examine the effect that an esthetic surface coating of NiTi archwires would have on the adherence of mutans streptococci. Material and method: three types of coated NiTi archwires (rhodium, gold and flexy blue) and one uncoated NiTi archwire with round cross section 0.016 inch were tested in the current study. Five pieces of each types of archwire were incubated in phosphate-buffered saline for two hours. Then, these wire segments were immersed for 5, 90, and 180 minutes with mutans streptococci suspension to conduct the bacterial adhesion assay. Result: there were statistically significant differences in the degree to which bacteria adhered to the various archwires at each time interval, with the level of adhesion being statistically highest on the uncoated NiTi wire and the level being statistically lowest on the gold-coated NiTi wire ($p < 0.05$). The result showed that the highest bacterial adhesion in 180 min followed by 90 min and the lowest in 5 min. A positive correlation was detected between bacterial adhesion and incubation time. Conclusion: the esthetic surface coating were influence the biofilm adhesion on the archwires, gold coated significantly decrease mutans streptococci adhesion when compare with uncoated NiTi wire. Higher incubation time increased the number of bacterial adherence for all types of archwires.

Introduction:

When orthodontic wire is placed, it has a tendency to develop new surfaces that are suitable for plaque production, which leads to an increase in the number of bacteria that are present in the oral cavity. Elements of orthodontic appliances such as brackets, archwires, and bands are used as plaque-retentive niches, which makes maintaining proper oral hygiene more difficult and results in a high cariogenic challenge(1, 2). Traditional orthodontic archwires are manufactured from stainless steel, cobalt-chromium-nickel alloy, nickel-titanium (NiTi), or titanium alloys. The Nickel titanium (NiTi) alloys are the most common aligning archwires(3).

The orthodontic archwire represents an important part of the fixed orthodontic appliance. The optimal archwire should have light, continuous force capability to move teeth. The goal of developing this force should be to reduce pain, hyalinization, and root resorption in the patient. The archwire should exhibit elastic behavior when subjected to force over a period of weeks to months(4). In recent times, there has been a notable increase in the demand for esthetic treatments, the patients' concerns regarding esthetics are not limited to the correction of the malocclusion; rather, they are also concerned with the appearance of the orthodontic appliance. The widespread availability of esthetically pleasing orthodontic materials has resulted in an increase in patients seeking orthodontic treatment(5). Most of the fixed orthodontic appliances are metallic and/or silver in color and are quite revealing to the outside environment(6). When brackets are manufactured, their appearance can be enhanced by using materials like ceramic or plastic. Alternately, the archwires' attractive color can be achieved by coating them in epoxy resin, teflon, rhodium, as well as a mixture of silver and biopolymers and 24K gold. The archwires will then have the desired color as a result of this(7). Therefore, there is a requirement for the availability of materials that have a higher level of aesthetics for patients, adequate clinical performance for clinicians, and the discovery of a new way to limit the adherence of bacteria(8).

During treatment, one of the most difficult aspects of orthodontics is ensuring that patients continue to practice good oral hygiene. Due to the difficulty in removing dental plaque from the teeth the braces, bands, and archwires can exacerbate these conditions, this can result in gingivitis and enamel demineralization, which can cause white spots and cavities(9). Streptococcus mutans is known to play an important role in the early stages of dental caries and enamel decalcification. This bacteria can be found in white-spot lesions where orthodontic products have been used(10). The process of enamel demineralization is attributed to the production of organic acids by *mutans streptococci* (MS), which includes S mutans and Streptococcus sobrinus. Hence, the degree of MS adhesion to orthodontic materials can be regarded as a crucial determinant in the advancement of enamel demineralization throughout the course of treatment.(11). The various surface properties of dental materials are what determine the amount of bacteria that will accumulate on those materials(12). High surface roughness values have a significant effect on bacterial adhesion because they reduce shear forces on initially adhering bacteria. Bacterial adherence is known to be enhanced by materials having high surface free energy values(11, 13). Few studies have expressed interest in the levels of bacterial adhesion to different types of orthodontic archwires in order to determine which material has the highest retention capacity of mutans streptococci. Previous research mainly focused on the physical and mechanical properties of components of fixed orthodontic appliances. For this important topic, we conducted the present study to quantitatively evaluate and compare the influence of flexy blue titanium, gold and rhodium coating of NiTi arch wires on mutans streptococci adhesion with respect to incubation time.

Material and Method

Experimental group's preparation

Four types of (NiTi) arch wire with round cross section (0.016 inches) three types coated and one type uncoated were used as shown in table (1).

Each arch wire type was divided into five segments measuring (24 ± 1) mm in length. The required sample size was five wire pieces for each time interval (5, 90 and 180) minutes, making the number of pieces for each kind of archwire 15 in each experiment. The experiment for each kind of archwire repeated three times making the total number of pieces 45 for each type. All of the wire segments were sterilized in an autoclave for twenty minutes at a temperature of 121 ºC and a pressure of 15 pounds per square inch(14).

Isolation of *Mutans Streptococci*

Pure isolates of *mutans streptococci* from stimulated saliva were used in all in vitro tests. Seven patients between the ages of 14 and 18 who appeared healthy volunteered for this project. Each person was instructed to chew a piece of paraffin gum (0.5 gm) for one minute, expectorate to remove any remnants of food, then chew the same piece of gum for another minute while saliva was collected in a sterile screw-top bottle(15). 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 distributed on MSB agar in duplicate using a sterile microbiological spreader. The plates were incubated anaerobically for 48 hours at 37°C with gas back (16). *S.mutans* was identified using cultural, morphological and biochemical characteristics as described by Slot 1992 (17).

Streptococci adhesion to different orthodontic archwire

Five segments of each type of arch wire were incubated in 2 ml of sterile phosphate-buffered saline (PBS, PH 7.2) at temperatures ranging from (25 to 30) °C for a period of two hours(18, 19). After that, each 5 pieces of arch wire were incubated at 37°C for different time interval (5, 90, and 180) minutes with agitation in a 5 ml suspension of bacteria at 10^7 -10^8 /ml. To get removal of any non-adherent microorganisms, the pieces of arch wire were immediately rinsed two times with PBS(20).

Culture of adhering *mutans streptococci* bacteria

For each experiment, the arch wire pieces with attached bacteria were washed with PBS and then the adhering bacteria were removed by treating the sample with 2 ml of a solution containing 0.25% trypsin/EDTA for 45 minutes at 37°C in an aerobic conditions (20) Fig (1). 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 experiment was performed in triplicate for each time, and the count that represented the average was determined.

Counting method:

In the current study, the plate counting technique, also known as the spread plate, was employed to determine the number of actively dividing and growing cells in a sample. This approach depends on bacteria's capacity to establish a colony on a nutritional medium that is visible to the naked eye(21).

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(22).

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

Statistical analysis:

All the data of the samples have been 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 tests and Pairwise comparison test were used for multiple comparisons. A value of $P<0.05$ was considered significant.

Result

Table (2) showed the amount of *streptococci mutans* adhesion to four types of archwires in three different incubation time. Kruskall-wallis H test used for comparison bacterial adhesion among the archwires, which showed there was statistically significant difference in bacterial adhesion among the archwires types. To compare between each two types of archwires using pairwise comparison test that showed a significant difference between uncoated NiTi and Gold coated archwire in all time interval Table (3).

Descrictive statistics Table (4) showed the amount of bacterial adhesion at different incubation time (5, 90 and 180) in different types of archwires. The result showed that the highest bacterial adhesion in 180 min followed by 90 min and the lowest in 5 min. kruskall-wallis H test used to compare the amount of bacterial adhesion among three time interval, which showed a significant difference with incubation time for all types of archwires. The result indicate that the longer incubation time significantly increase the bacterial adhesion in all types of archwires Fig (2).

Discussion

It is well-known that orthodontic archwires make teeth cleaning more difficult and increase the adhesion sites for oral pathogens like *Streptococcus mutans*(23). The colonization of *cariogenic mutans streptococci* has a significant role in the development of enamel demineralization associated with orthodontic appliances(24).

Understanding how cariogenic streptococci develop and attach to orthodontic appliances will lead to more effective measures against enamel demineralization and white spot formation(25).

Bacterial adhesion to a surface is a dynamic process that depends on numerous factors including the bacterial species and the surface of the material.

Archwires used in orthodontic treatment can be made from a variety of metal alloys, have a range of mechanical characteristics and SR values, and even have the potential to be coated with specific substances to enhance their esthetic appearance while the patient is undergoing treatment(26).

This research focused on the esthetic benefits of various archwire coatings that are currently being explored to enhance the qualities of dental materials, which are also seeing increased use in orthodontics. Due to the fact that there are more bacteria present during orthodontic treatment, the purpose of this study is to investigate the impact that esthetic coatings have on the total amount of bacteria that become deposited on orthodontic archwires. According to research, an increase in the number of bacteria can also have an effect on the characteristics of orthodontic archwires(27).

According to the results of this investigation, there was a detectable variation in the amount of bacterial adherence that occurred with each surface coating, multiple comparisons revealed that the uncoated wires had higher *streptococci mutans* adhesion than esthetic coated wires with statistical significant difference when compared with gold coated wire in all time interval. It is reasonable to expect that the different surface properties for the various types of archwire will result in variable levels of bacterial adherence. Surface roughness and surface free energy are two surface properties that have an effect on the degree to which bacteria adhere to the surface of orthodontic material(28).

The higher adhesion rates on uncoated wires might be attributed to the surface roughness of the archwire. The roughness of uncoated NiTi was found to be greater than that of coated NiTi in a study that was carried out by D'Antò, et al.(29) and Mousavi, et al.(30).

Lee, et al. (11) assessed the surface properties of orthodontic materials and examined the impact of these properties on the adherence of *Streptococcus mutans*. According to their findings, an increase in surface roughness and surface energy is associated with a greater degree of absorption of *Streptococcus mutans*. The researchers reached the conclusion that the increase in surface area and retention sites linked to surface roughness results in elevated plaque buildup. Additionally, they indicated that alterations in surface energy disrupt acid-alkaline and van der Waals interactions, which play a crucial role in the initial adhesion of bacteria which is consistent with the data obtained in this study.

The grain re-crystallizations that take place as a result of pulling NiTi wires through diamond molds, which is one step in the manufacturing process, are the primary cause of this product's extremely rough surface(31).

Rough surface offer chance for bacterial adherence by increasing the exposed surface area, producing appropriate bacterial adhesion and weakening bacterial colony displacement(32)

The lower adhesion of bacteria on gold coated wire may be attributed to the reduced surface free energy exhibited by the gold coating. Materials with lower surface free energy exhibit reduced bacterial adhesion compared to those with higher surface free energy (28, 33). It has been found that during processing of gold-plated archwires, uneven cooling at elevated temperatures can result in internal structural rearrangements accompanied by volume fluctuations. These changes induce internal stress, which in turn may give rise to cracks in the outer protective layer and accelerate corrosion, ultimately leading to an increase in surface roughness (34, 35). Study conducted by kim, et al. (36) on gold coated wire showed that the low surface free energy of this wire is responsible about the decrease of *mutans streptococci* on it. This suggests that the adherence of *mutans streptococci* is not only determined by surface roughness. It is widely recognized that alterations in surface roughness have a significant impact on the contact area, thereby leading to variations in surface free energy measurements.

The amount of bacterial adherence on rhodium coated slightly lower than uncoated NiTi but statistically not significant. Which was in agreement with Asiry, et al.(37), and Oliveira, et al. (38) that showed rhodium coated and uncoated wires were categorized as medium roughness comparable with other types of archwire. This similarity in roughness may explain the absence of statistically significant differences in bacterial adhesion between these two materials, as observed in this investigation. This finding disagrees with the outcomes of a randomized clinical trial conducted by Lima et al. (32) that demonstrated the application of rhodium coating results in an increase of bacterial adhesion.

Flexyblue-NiTi archwire was treated by oxidation under high temperature to enhance the properties that change the color of archwire to light blue. The oxide layer on the alloy’s surface becomes thicker and increase with increasing oxidation temperature and time(39).

No previous study of this type of archwire regarding bacterial adhesion and from the result of current study found that no significant difference when compared with uncoated NiTi.

Regarding the effect of incubation time, higher incubation time increased the number of bacterial adherence for all types of archwires; the number of adhesion was highest after 3 hours of incubation than 5 and 90 minutes, this may be in agreement with the findings of Al-Lami and Al-Sheakli(40)and Jasim, et al. (41) who discovered that a longer incubation time increases the bacterial adherence.

Conclusion

- Some esthetic surface coating archwires significantly decrease *S. mutans* adhesion compared to uncoated archwires.
- Positive correlation was detected between incubation time and the amount of *S. mutans* adhesion.

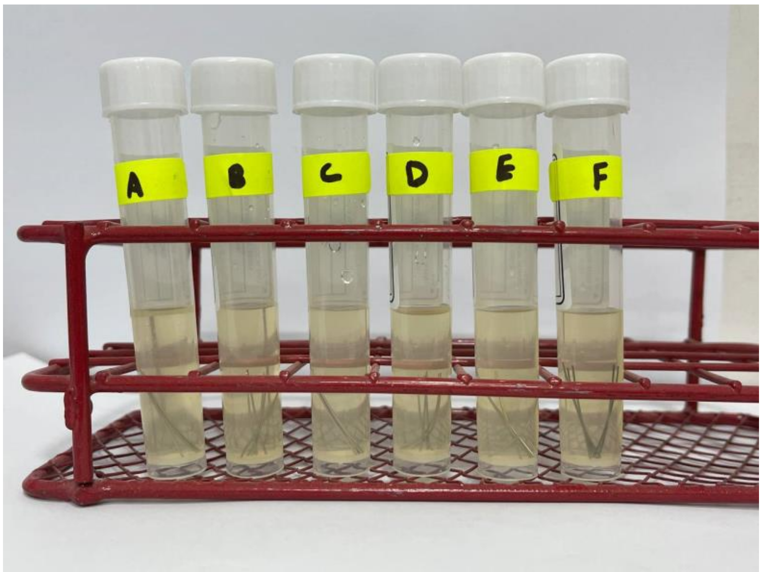

Figure (1): treating the sample with trypsin/EDTA

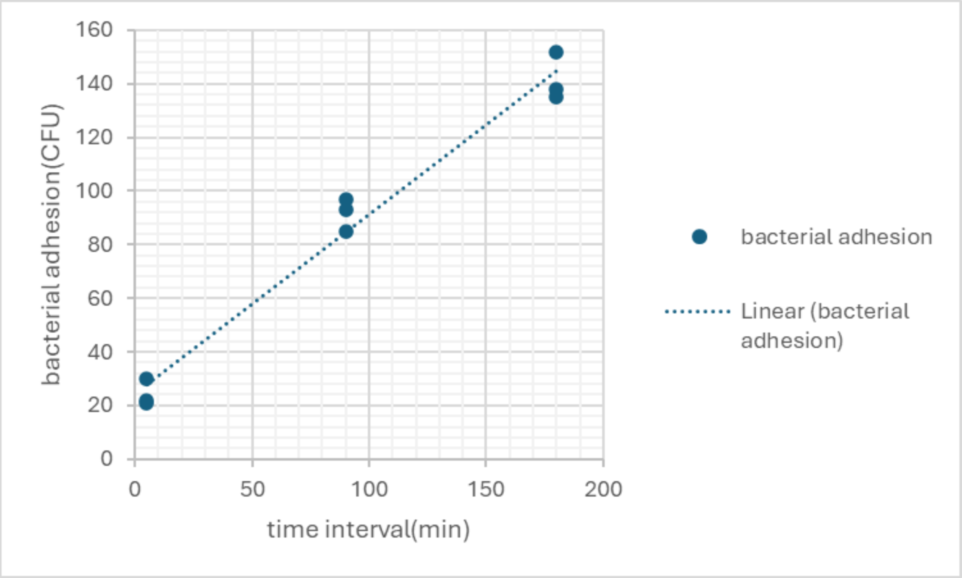

Figure (2) correlation between bacterial adhesion and incubation time

Table (1): investigated orthodontic arch wires in the current study

Name of arch wire	Manufacturer
Nickel-titanium alloy	Orthometric company
Flexyblue nickel-titanium	Orthometric company
Rhodium coated nickel-titanium	IOS company
Gold coated nickel-titanium	IOS company

Table (2): Descriptive statistic and Comparison No. of adherent mutans streptococci in each time intervals on different type of archwire

Time	Group	Descriptive statistic					Groups' comparison (kruskall-wallis H test)	
		Mean	Median	SD	Max	Min	X^2	p-value
5 min	NiTi	24.3	22	4.93	30	21	9.4	0.024 (S)
	Flexyblue	6.6	6	2.08	9	5		
	Rhodium	6.6	6	3.05	10	4		
	Gold	2.6	3	0.57	3	2		
90 min	NiTi	91.6	93	6.11	97	85	9.6	0.022 (S)
	Flexyblue	12.3	12	2.51	15	10		
	Rhodium	22	25	9.84	30	11		
	Gold	7.6	8	1.52	9	6		
180 min	NiTi	141.6	138	9.07	152	135	9.8	0.020 (S)
	Flexyblue	25.6	23	7.37	34	20		
	Rhodium	33.3	34	3.05	36	30		
	Gold	10.3	10	1.52	12	9		

Table (3): Comparisons between each two types of archwires using pairwise comparison.

Time	Groups	Pairwise comparison
5 min	(NiTi-Flexyblue)	0.125
	(NiTi-Rhodium)	0.125
	(NiTi-Gold)	0.002 (HS)
	(Flexyblue-Rhodium)	1.0
	(Flexyblue-Gold)	0.125
	(Rhodium-Gold)	0.125
90 min	(NiTi-Flexyblue)	0.07
	(Niti-Rhodium)	0.213
	(NiTi-Gold)	0.002 (HS)
	(Flexyblue-Rhodium)	0.571
	(Flexyblue-Gold)	0.213
	(Rhodium-Gold)	0.07
180 min	(NiTi-Flexyblue)	0.061
	(NiTi-Rhodium)	0.234
	(NiTi-Gold)	0.002 (HS)
	(Flexyblue-Rhodium)	0.496
	(Flexyblue-Gold)	0.234
	(Rhodium-Gold)	0.061

Table (4): Descriptive statistic and comparison No. of adherent mutans streptococci in each time intervals on different type of archwire

Type of archwire	Time	Descriptive statistic					Durations' comparison				
							Kruskall-wallis H-test		Pairwise comparison test		
		Mean	Median	SD	Max	Min	X^2	p-value	5-90	5-180	90-180
NiTi	5 min	24.3	22	4.93	30	21	7.2	0.027 (S)	0.18 (NS)	0.007 (S)	0.18 (NS)
	90 min	91.6	93	6.11	97	85					
	180 min	141.6	138	9.07	152	135					
Flexyblue	5 min	6.6	6	2.08	9	5	7.2	0.027 (S)	0.180 (NS)	0.007 (S)	0.180 (NS)
	90 min	12.3	12	2.51	15	10					
	180 min	25.6	23	7.37	34	20					
Rhodium	5 min	6.6	6	3.05	10	4	6.8	0.032 (S)	0.155 (NS)	0.009 (S)	0.231 (NS)
	90 min	22	25	9.84	30	11					
	180 min	33.3	34	3.05	36	30					
Gold	5 min	2.6	3	0.57	3	2	6.9	0.031 (S)	0.153 (NS)	0.009 (S)	0.229 (NS)
	90 min	7.6	8	1.52	9	6					
	180 min	10.3	10	1.52	12	9					

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