Quantitative assessment of Mutans Streptococci adhesion to coated and uncoated orthodontic archwires (In vitro study)

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ABSTRACT

Background: The development of orthodontic biomaterials that attract less biofilm has been a goal for decades. Adhesion and colonization of cariogenic streptococci are considered to play key roles in the development of enamel demineralization related to orthodontic materials. The aim of this study was to quantitatively evaluate the Mutans streptococci adhesion to coated orthodontic archwires (Epoxy and Teflon) and uncoated archwires (stainless steel and nickel-titanium) with respect to incubation time in the presence and absence of saliva.

Material and Method: Six types of archwires stainless steel and nickel titanium with two type of coating (Epoxy, Teflon) were used in this study. Twelve specimens of each archwire were incubated in sterilized unstimulated whole saliva (for the study group) and phosphate-buffered saline (for control group) for 2 hours, then incubated with suspension of Mutans streptococci allowed to adhere for (5,90,180 minutes). Adhesion was quantitated by a microbial culture technique by treating the archwires with adhering bacteria with trypsin and enumerating the colony forming unit (CFU) counts of bacteria recovered after cultivation by using Dentocult SM kit.

Results: There was significant difference among the tested archwire types in each time interval with the highest bacterial adhesion on the NiTi archwires in the absence of saliva. In the presence of saliva, the results revealed non-significant difference at 5 min. while there was significant difference at 90 min and highly significant difference at 180 min.

Conclusion: The adherence of Mutans streptococci was decreased in the presence of saliva on different archwires and the extended incubation time was significantly related to increase colony forming unit of Mutans Streptococci.

Keyword: Mutans Streptococcus, coated orthodontic archwires. (J Bagh Coll Dentistry 2014; 26(4):156-162).

INTRODUCTION

The development of orthodontic biomaterials (OB) that attract less biofilm has been a goal for decades, but is hampered by alack of knowledge of the fundamental aspects of bacterial adhesion to the different OB materials [(1,2)]. The oral environment provides the proper conditions for the colonization of a complex microbiota [(3)]. In a healthy oral cavity, these microorganisms coexist in a balanced state with their host. But when changes occur in the normal oral environment, the balanced flora changes and imbalance and disease may result [(4)].

Although a large number of studies have shown a shift in microbial populations in the presence of orthodontic fixed appliances, limited information is available as to which material would be less prone to adhesion of bacterial species and plaque accumulation [(5,6)].

Adhesion and colonization of cariogenic streptococci are considered to play key roles in the development of enamel demineralization related to orthodontic materials [(7)], because these materials in the oral cavity present a unique surface that can interact with bacteria, leading to pathogenic plaque formation for enamel demineralization [(6,8)].

Several studies reported that the placement of fixed orthodontic appliances leads to increases in the volume and number of cariogenic streptococci in dental plaque, and the elevated levels of streptococci return to normal after removal of the appliance [(9-11)].

Most of the previous studies were concerned with mechanical aspect of component of fixed orthodontic appliances [(12,13)] and there is no study concerning the levels of adhesion of cariogenic streptococci to various types of orthodontic archwires to determine which material has a higher retention capacity of Mutans streptococcus.

The aim of this study was to quantitatively evaluate the Mutans streptococci adhesion to coated orthodontic archwires (Epoxy and Teflon) and uncoated archwires (stainless steel and nickel-titanium) at different incubation time in the presence and absence of saliva coating.

MATERIALAND METHODS

Specimen preparation

Six types of commercially available archwire with round sections (0.018 inches) of different materials (stainless steel and nickel titanium) with and without coating were tested as shown in (table 1).

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Table 1. Orthodontic archwires investigated in this study

Name of archwire	Manufacturer
OrthoForce S304vm stainless steel	G&H Wire Company
SE NiTi	G&H wire Company
Stainless steel Tooth coated	G&H Wire Company
OrthoForce Ultraesthetic G4 NiTi Tooth Coated	G&H Wire Company
Dany SS coated Archwire	DANY BMT Company
OrthoForce Ultraesthetic G4 NiTi –poly Tooth Coated	G&H Wire Company

Each type of the ready-made wire was cut into 6 pieces of (20±1) mm. The suggested sample was to have 12 wire-pieces per each subgroup, making the total (216) pieces. All of the wire-pieces were sterilized by autoclave at 20 minute at 121 ºC at 15 pound [14,15].

Isolation of Mutans Streptococci

In vitro experiments were all carried out using pure isolate of Mutans streptococci from stimulated saliva collected. A five healthy looking patients aged (14-18) years were volunteered for this purpose. Saliva sample collected according to Thylstrup and Fejerskov [16]. Vortex mixer homogenized each saliva for two minutes. Ten fold serial dilutions were performed by transferring 0.1 ml to 0.9 ml sterilized normal saline. From dilution 10^{-2} and 10^{-4} of salivary samples 0.1 ml was taken and spread in duplicate on the Mitis salivaris Bacitracin agar. These plates were incubated anaerobically using gas back, incubation period was for 48 hours at 37º C, and then plates were incubated aerobically for 24 hours at 37º C [17].

Unstimulated saliva collection

Saliva was collected from three volunteers a 35-year-old man of good oral health who had refrained from eating, drinking, and brushing for at least 2 hours before saliva collection. These volunteers had no initial dental caries and periodontal lesions. Saliva collection was performed from 7:00 to 8:00 AM to minimize the effects of diurnal variability in salivary composition according to Ahn et al. and Yang et al. [10,18]. The pH of saliva was roughly determined by using sensitive pH paper and saliva that showed pH out of the acceptable range (6.5-7.2) was excluded from the experiments.

Adhesion of streptococci to orthodontic archwire

Twelve specimens of each type of archwire were incubated in 2 ml of UWS with agitation for 2 hours at air-conditioned room (25-30 ºC). For negative control tests, the same procedure was performed with sterile phosphate-buffered saline (PBS, PH 7.2) instead of UWS [10,19]. The specimens were washed 3 times with phosphate-buffered saline solution. The specimens incubated in 5 ml suspension of bacteria at 10^{7}-10^{8} /ml with agitation for (5, 90 and 180 minutes) at 37º C. Afterwards, the specimens were rinsed 2 times immediately carefully with PBS to remove any non-adherent bacteria [20].

Culture of adhering bacteria

For each experiment, after the washing with PBS, the specimens with their adhering bacteria from each tube were treated with 2 ml of 0.25% trypsin/EDTA for 45 minutes in aerobic conditions at 37º C , for detachment of the adherent bacteria [20].

The Kit of Dentocult SM Strip Mutans (Orion Diagnostica) was used to detect S. Mutans for in vitro diagnostic only. The method is based on the use of selective culture and growth of S. Mutans on the test strip.

Strips were inserted in these solutions for five minutes. The bacitracin discs were placed in the selective culture vials 15 minutes before, and then strips were transferred to these vials and incubated for 48 hours at 37º C. The final step was the counting of adherent bacteria on the strips, and the number of colony-forming unit (CFU)/strip [21] **(Figure1)**. Experiment in each time was made in triplicate and the average count was determined.

Figure 1: Detection of MS on Dentocult strip.

Statistical analyses

Data was presented in simple statistical measure of number, median, mean, standard deviation, and standard error. Statistical analysis was done by using Mann-Whitney U tests and Kruskall-Wallis H test A probability value ($P< 0.05$) was considered to be statistically significant.

RESULTS

Table 2 showed the number of adherent bacteria on different type of archwire in various time intervals without saliva. Generally, there was significant difference among the tested archwire types in each time interval with the highest bacterial adhesion on the NiTi archwires.

Table 3 demonstrated the comparison between each two types of archwires with almost significant difference.

Table 4 showed the number of adherent bacteria on different type of archwire in various time intervals with saliva. The results revealed non-significant difference at 5 min. while there was significant difference at 90 min and highly significant difference at 180 min.

Again table 5 illustrated the comparison between each two types of archwires with mostly significant difference except the comparison between epoxy NiTi with Teflon coated archwires and NiTi with epoxy archwires in 90 min.

DISCUSSION

Patients faced difficulty in maintaining adequate oral hygiene when wearing fixed orthodontic appliances [22]. The increased plaque accumulation and bacterial acid production result in enamel decalcification and even inflammation of the surrounding periodontal tissues [23, 24].

Orthodontic archwires play a significant role in plaque accumulation; therefore, awareness of the bacterial adhesion tendency of the new orthodontic archwire materials should be developed in order to select an archwire type that attracts less biofilm and has appropriate anti-bacterial properties.

The finding of this study proved that all archwires coated with saliva were associated with decrease number of adherent bacteria in different duration in comparison with sample without saliva. These findings indicate that saliva is an important factor in the adhesion of Mutans streptococci to orthodontic archwires. This may be explained by the formation of an early salivary pellicle result in reduction of the bacterial adhesion to the archwires [25,26] ,on the contrary with the non–saliva coated archwires [20,26-30].

Additionally, the presence of histatins, lyzozymes and lactoperioxidase components of the saliva, which possess exceptional antibacterial activities, may also contribute to the decreased adhesion of S. Mutans to saliva treated archwires in vitro [31-33].

Contrary to the present findings, Ahn et al. [42-43] found that saliva coating did not significantly influence the adhesion of bacteria to orthodontic brackets which explained that saliva coating reduces the surface free energy of the underlying materials.

The result of the present study revealed that in multiple comparisons, the highest adhesion of cariogenic streptococci on NiTi and Epoxy coated while lowest for the stainless steel and Teflon coated materials. This could be explained by a study conducted by Amini et al. and D' Anto et al. [34,35] which showed that roughness of NiTi is responsible for the increase in the count of cariogenic streptococci. A study on Epoxy coated proved that the same reason (roughness of he surface) [36] is responsible for the increase in the colonization of cariogenic bacteria.

Studies on stainless steel wires [37,38] showed that the smoothness of the surface is responsible about the decrease of colony count of Streptoccus on it.

The presence of fluoridated chain in Teflon coated archwires, which is responsible for its chemical and physical characteristics will explain the lowest colony count of Mutans streptococci according to some studies [39-41].

This study highlighted the role of the incubation time in modulating adhesion of cariogenic streptococci. The adhesion in the coated and non-coated group was increased by the extended incubation time and was the highest after three hours of incubation. These findings agreed with other studies of which found that extended incubation time increased the adhesion of cariogenic Mutans streptococci [10,42,43].

Table 2: Comparison No. of adherent bacteria on different type of archwire in each time without saliva

Duration	Groups	Descriptive Statistics				Groups'comparison (Kurskall-Wallis H test)	
		Median	Mean	S.D.	S.E.	X^2	p-value
5min	SS	7	6.67	1.53	0.88	12.25	0.032 (S)
	NiTi	17	16.67	3.51	2.03		
	E-SS	10	9.67	2.52	1.45		
	E-NiTi	10	9.33	2.08	1.20		
	T-NiTi	6	5.67	0.58	0.33		
	T-SS	7	7	2	1.15		
90min.	SS	65	65	5	2.89	15.76	0.008 (HS)
	NiTi	105	106.33	8.08	4.67		
	E-SS	85	85	5	2.89		
	E-NiTi	88	87.67	2.52	1.45		
	T-NiTi	73	74.33	3.21	1.86		
	T-SS	76	75.67	3.51	2.03		
180min	SS	127	129.00	5.29	3.06	14.88	0.011 (S)
	NiTi	170	171.33	3.21	1.86		
	E-SS	150	151.67	3.79	2.19		
	E-NiTi	145	148.33	8.50	4.91		
	T-NiTi	135	134.67	3.51	2.03		
	T-SS	130	132.67	6.43	3.71		

1- SS= stainless steel; 2- NiTi= nickel-titanium; 3- E-SS= Epoxy coated stainless steel; 4- E-NiTi= Epoxy coated nickel-titanium; 5- T-NiTi= Teflon coated nickel-titanium; 6- T-SS= Teflon coated stainless steel.

Table 3: Comparisons between each two groups using Mann-Whitney U test

Groups		5minutes P-value	90minutes P-value	180minutes P-value
SS	NiTi	0.05*	0.05*	0.05*
	E-SS	0.184	0.05*	0.05*
	E-NiTi	0.184	0.05*	0.05*
	T-NiTi	0.369	0.05*	0.184
	T-SS	0.822	0.05*	0.275
NiTi	E-SS	0.05*	0.05*	0.05*
	E-NiTi	0.05*	0.05*	0.05*
	T-NiTi	0.046*	0.05*	0.05*
	T-SS	0.05*	0.05*	0.05*
E-SS	E-NiTi	0.822	0.5	0.513
	T-NiTi	0.046*	0.05*	0.05*
	T-SS	0.184	0.05*	0.05*
E-NiTi	T-NiTi	0.046*	0.05*	0.05*
	T-SS	0.184	0.05*	0.05*
T-NiTi	T-SS	0.369	0.658	0.513

Table 4: Comparison No. of adherent bacteria on different type of archwire in each time with saliva

Duration	Groups	Descriptive Statistics				Groups' comparison (Kurskall-Wallis H test)	
		Median	Mean	S.D.	S.E.	X^2	p-value
5 min.	SS	5	4.67	1.53	0.88	9.64	0.086 (NS)
	NiTi	10	10.67	4.04	2.33		
	E-SS	4	4.33	1.53	0.88		
	E-NiTi	3	3.33	0.58	0.33		
	T-NiTi	4	3.33	1.15	0.67		
	T-SS	3	3	1	0.58		
90 min.	SS	43	42.33	3.06	1.76	13.63	0.018 (S)
	NiTi	70	70.67	6.03	3.48		
	E-SS	65	65	5	2.89		
	E-NiTi	68	63.33	9.87	5.70		
	T-NiTi	48	50.33	5.86	3.38		
	T-SS	52	52.67	3.06	1.76		
180 min	SS	95	93.67	5.13	2.96	16.03	0.007 (HS)
	NiTi	148	148	8	4.62		
	E-SS	120	120	5	2.89		
	E-NiTi	109	109.33	7.51	4.33		
	T-NiTi	89	90	2.65	1.53		
	T-SS	74	75.33	3.21	1.86		

Table 5: Comparisons between each two groups using Mann-Whitney U test

Groups		90minutes P-value	180minutes P-value
SS	NiTi	0.05*	0.05*
	E-SS	0.05*	0.05*
	E-NiTi	0.05*	0.05*
	T-NiTi	0.05*	0.376
	T-SS	0.05*	0.05*
NiTi	E-SS	0.216	0.05*
	E-NiTi	0.376	0.05*
	T-NiTi	0.05*	0.05*
	T-SS	0.05*	0.05*
E-SS	E-NiTi	1	0.127
	T-NiTi	0.05*	0.05*
	T-SS	0.05*	0.05*
E-NiTi	T-NiTi	0.127	0.05*
	T-SS	0.184	0.05*
T-NiTi	T-SS	0.513	0.05*

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