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Differences in the Microbial Colonization Among Arch Wire Types, Gauges and Cross Sections

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ABSTRACT

Background: *The presence of orthodontic materials in the oral cavity represent a unique surface that can interact with bacteria, leading to pathogenic plaque formation and subsequent enamel demineralization, Streptococcus mutans play an important role in the initiation and progression of dental caries and they are considered the primary cause of bacteriological caries. The objective of this study was to investigate the effect of multiple factors including the type of arch wire, salivary coating, cross section, and wire thickness on the levels of mutans streptococci adherence.* ***Materials and Methods:*** *Two types of arch wire stainless steel and nickel titanium were selected using the following criteria: round and rectangular with gauges 0.014, 0.018, 0.016 × 0.022 and 0.019 × 0.0 25 inches which were subdivided into eight groups. Bacterial adhesion was quantified by a microbial culture technique and the number of adhesive bacteria were analyzed and counted after growth in culture for each group with and without saliva coating at 15 and 60 minutes. Detection of mutans streptococci by saliva-check Mutans test.* ***Results:*** *There was a significant difference between arch wire types in each time interval and the highest bacterial adhesion on the NiTi arch wires with rectangular cross section in the absence of saliva with extended incubation time.* ***Conclusions:*** *The adherence of mutans streptococci in saliva coated wires seems to be low. At increased incubation time, rectangular cross section arch wire showed an increased number of adhering bacteria with less effect on different gauges of the arch wire.*

Keywords: Gauge of arch wire, Cross section of arch wire, Microbial colonization

INTRODUCTION

Enamel demineralization associated with orthodontic materials is considered to be related significantly to the adhesion and colonization of cariogenic bacterial species. Presence of these materials in the oral cavity represent a perfect environment to the bacteria which can interact with them leading to pathogenic plaque formation and subsequent enamel demineralization. The increased demands for super elasticity, corrosion resistance and biocompatibility lead to the progression in orthodontic arch wire materials from stainless-steel and cobalt chromium-nickel alloys to the nickel-titanium (NiTi) alloy [1,2].

Several studies proved that the placement of fixed orthodontic appliances can increase volume and number of the cariogenic streptococci in dental plaque [3-5]. Therefore, availability of materials that have a greater esthetics for the patients, suitable clinical performance for clinicians and find a new solution to reduce the bacterial adhesion is needed. This problem was resolved by the introduction of esthetic brackets made of ceramic or composite [6].

Although wires play a significant role in enamel demineralization when used during the period of orthodontic treatment, adhesion and colonization of cariogenic streptococci that interact with these surfaces followed by pathogenic plaque formation are considered the main reasons of developing this process [5].

Areas of contact between wires and brackets provide a unique environment that impedes proper access to the tooth surfaces for cleaning; rougher surfaces permitted bacterial colonization and increase the adhesion areas [6]. Eliades, et al. [7] found that stainless steel represents the highest percentage of surface tension and energy and is expected to have higher plaque retaining capacity.

Most previous studies focused on the physical and mechanical properties of component of fixed orthodontic appliances but there are few studies that have expressed interest concerning levels of adhesion of cariogenic streptococci to various types of orthodontic arch wires to find out which material has highest retention capacity of *mutans streptococci* and for this important topic we have conducted the present study.

The objective of this study was to investigate the effect of multiple factors including the type of arch wire, salivary coating, cross section, and wire thickness on the levels of *mutans streptococci* adherence.

MATERIAL AND METHODS

Sample

Two types of commercially available arch wire stainless steel and nickel titanium from Morelli Company with round sections (0.014, 0.018) inches and rectangular section (0.016 × 0.022, 0.019 × 0.025) inches were tested. One hundred sixty specimens of orthodontic wires from Morelli Company were tested. Each type of wire was cut into 8 pieces of 15 ± 1 mm. The suggested idea was to have 20 wire-pieces per each subgroup, the total will be 160 pieces.

Groups were divided as follows: Stainless steel 0.014 inch (Group A), stainless steel 0.018 inch (Group B), stainless steel 0.016 × 0.022 inch (Group C), stainless steel 0.019 × 0.025 inch (Group D), nickel titanium 0.014 inch (Group E), nickel titanium 0.018 inch (Group F), nickel titanium 0.016 × 0.022 inch (Group G), and nickel titanium 0.019 × 0.025 inch (Group H).

Isolation of *Streptococcus mutans*

Stimulated whole saliva samples were collected under standard conditions from three healthy volunteers aged (22-25) years.

For isolation of *Streptococcus mutans*, each individual was instructed to chew a piece of Arabic chewing gum (0.4-0.5 g) for five minutes to stimulate salivary flow as much as possible [8].

Methods of isolation and identification of *mutans streptococci* were according to those described by Holbrook and Beighton [9] and Finegold and Baron [10]. The isolation of MS was done in teaching laboratories of Baghdad medical city. Saliva was collected in sterilized screw capped bottles. The collected saliva was homogenized by vortex mixer for two minutes. Tenfold serial dilution was 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 withdrawn and spread in duplicate, by using sterile microbiological glass spreader, on the selective mitis salivarius-bacitracin (MSB) agar medium prepared according to the manufacturer's instructions. These plates were incubated anaerobically using gas pack, incubation period was for 48 hours at 37°C, and then plates were incubated aerobically for 24 hours at 37°C [11]. A single colony from *mutans streptococci* was transferred to 10 ml sterile Brain Heart Infusion Broth (BHI-B) and then incubated aerobically for 18 hours at 37°C to activate the inoculums. The purity of the isolates was checked by inoculation of 0.1 ml of the isolates from BHI-B suspensions on media by spreader as mentioned before, and then a selective colony was transferred to 10 ml of sterile BHI-B and incubated aerobically for 24 hours at 37°C.

Identification of *mutans streptococci* was carried out by 4 stages:

a) Colony morphology.

b) Morphological test of bacterial cell.

c) Biochemical test.

d) Identification system for *mutans streptococci* of Analytic Profile Index (API) 20 strep.

Maintenance of the microbial isolates were done, tubes with broth were stored in the refrigerator till use. Bacterial activation was done before each experiment by addition of 0.1 ml pure isolates of MS to 5 ml of sterile Tryptose-Phosphate broth incubated aerobically for 18-24 hours at 37°C.

Unstimulated Whole saliva (UWS) was collected from two volunteers with good oral health with exclusion of initial dental caries and periodontal lesions, UWS was collected in a sterile tube by spitting method then the sample centrifuged for 10 minutes. The supernatants layer used immediately after filter sterilization [3,12].

Specimens of each type of arch wire were incubated in 2 ml of UWS with agitation for two hours at air-conditioned room 25°C to 30°C. For negative control tests, the same procedure was performed using sterile phosphate- buffered saline (PBS-, pH 7.2) instead of UWS [3,13].

The specimens were washed three times with phosphate buffered saline solution, then incubated in 10 ml suspension of bacteria at 10^7 - 10^8 dilutions with agitation for (15 and 60 minutes) at 37°C. Afterwards, the specimens were rinsed 2 times immediately and carefully with PBS to remove any non-adherent bacteria [14].

For each experiment, after washing with phosphate-buffered saline, 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 37°C, for detachment of the adherent bacteria [14].

Detection of *mutans streptococci*

Saliva-Check Mutans test (GC/Tokyo-Japan) provides easy detection of *mutans streptococci* from a saliva sample. Saliva-Check *Mutans* uses a very specific immunochromatography process, not reliant on bacteria growth. This means incubators or other devices are not needed and accurate results are available in just 15 minutes.

The test strip contains 2 monoclonal antibodies that selectively detect only the *Streptococcus mutans* species, meaning no other bacteria contaminate the results. The final step was the counting of adherent bacteria on the strips, and the number of colony-forming unit (CFU)/strip.

Statistical Analysis

Data were collected and statistically analyzed by a software computer program SPSS (Statistical Package of Social Science) software version 15 for windows XP. The following statistics were used:

- **Descriptive Statistics**

Mean, and standard deviation.

- **Inferential Statistics**

1. **Mann-Whitney U test:** To compare the bacterial adhesion between each two groups of arch wire for each cross section, saliva covering, nickel titanium and stainless-steel arch wire.
2. **Kruskal-Wallis test:** To test any statistically significant difference of the bacterial adhesion among the four groups of the same arch wire material at each duration.

P>0.05 was considered as non-significant (NS); $0.05 \geq P > 0.01$ was considered as significant (S) and; $P \leq 0.01$ as highly significant (HS).

RESULTS

The descriptive statistics (Table 1) and Mann-Whitney U test which is used for comparison of the mean value of bacterial colonies adhesion for each group A, B, C, D, E, F, G, and H separately between two condition (with saliva and without saliva) at each duration 15 and 60 minutes, that showed highly significant difference in bacterial adhesion between two condition (with saliva and without saliva).

Table 1 Comparison of the number of adherent bacteria MS on stainless steel and NiTi arch wires between two conditions with and without saliva in each duration

Incubation time		15 minutes				60 minutes			
Groups		Mean	Std. Deviation	Mann Whitney U	p-value	Mean	Std. Deviation	Mann-Whitney U	p-value
A	With and Without Saliva	26.53	18.918	0.00	0.000**	56.88	8.456	0.00	0.000**
B	With and Without Saliva	27.45	19.508	0.00	0.000**	57.15	8.066	0.00	0.000**
C	With and Without Saliva	40.65	28.186	0.00	0.000**	70.98	8.182	0.00	0.000**

D	With and Without Saliva	17.35	4.933	0.00	0.000**	70.9	8.883	0.00	0.000**
E	With and Without Saliva	11.8	2.972	35	0.000**	55.5	11.266	0.00	0.000**
F	With and Without Saliva	12.58	3.137	69.5	0.000**	56.83	11.27	0.00	0.000**
G	With and Without Saliva	22.85	3.446	65	0.000**	73.65	6.503	7.5	0.000**
H	With and Without Saliva	23.88	4.058	57.5	0.000**	75.78	6.294	34	0.000**

The descriptive statistics (Table 2) and Mann-Whitney U test which is used for comparison of the mean value of bacterial adhesion between each two groups of the same arch wire material and the same cross section but different in wire gauge at each duration 15 and 60 minutes, which showed non-significant statistical difference in bacterial adhesion between each two groups of the same arch wire material, except between these groups A and B without saliva at 15 minutes and G and H with saliva at 60 minutes showed significant difference.

Table 2 Comparison of the number of adherent bacteria MS between different gauges of stainless steel and NiTi arch wires in two conditions in each duration

Incubation time		**15 minutes**				**60 minutes**			
Groups		**Mean**	**Std. Deviation**	**Mann Whitney U**	**p-value**	**Mean**	**Std. Deviation**	**Mann-Whitney U**	**p-value**
A&B	Without Saliva	11.05	1.552	122	0.032*	64.65	3.026	175.5	0.505 (NS)
	With Saliva	8.25	2.157	179	0.566 (NS)	49.38	2.789	166.5	0.362 (NS)
C&D	Without Saliva	21.78	1.687	168	0.377 (NS)	78.45	3.551	193.5	0.859 (NS)
	With Saliva	12.98	2.38	198.5	0.967 (NS)	63.43	4.181	182.5	0.634 (NS)
E&F	Without Saliva	14.08	2.795	190	0.785 (NS)	66.6	4.162	176.5	0.524 (NS)
	With Saliva	10.3	1.964	145	0.132 (NS)	45.73	3.748	163	0.315 (NS)
G&H	Without Saliva	25.63	3.746	163	0.315 (NS)	79.55	4.218	175	0.496 (NS)
	With Saliva	21.1	2.085	174.5	0.486 (NS)	69.88	4.292	127	0.048*

The descriptive statistics (Table 3) and Mann-Whitney U test used for comparison the mean value of bacterial adhesion between each two groups of the same wire gauge and the same cross section but different in arch wire material at each duration 15 and 60 minutes, which showed highly significant difference in bacterial adhesion between each two groups of the same wire gauge.

Table 3 Comparison of the number of adherent bacteria MS between stainless steel and NiTi archwires

Group		**15 minutes**				**60 minutes**			
		Mean	**Std. Deviation**	**Mann-Whitney U**	**P-value**	**Mean**	**Std. Deviation**	**Mann-Whitney U**	**P-value**
A and E	Without Saliva	12.18	2.659	53	0.000**	65.43	3.396	95	0.002**
	With Saliva	8.9	2.098	108	0.012*	46.95	3.58	74.5	0.001**
B and F	Without Saliva	12.95	2.745	87	0.002**	65.83	4.101	101	0.003**
	With Saliva	9.65	2.445	85	0.002**	48.15	3.887	100.5	0.004**
C and G	Without Saliva	24.1	3.808	63	0.000**	78.55	4	73	0.000**
	With Saliva	16.88	4.547	0	0.000**	66.08	4.135	1	0.000**
D and H	Without Saliva	24.03	3.952	1	0.000**	79.45	3.823	102	0.003**
	With Saliva	17.2	4.81	88	0.000**	67.23	6.282	33	0.000**

The descriptive statistics (Table 4) and Kruskal-Wallis test used for comparison of the mean value of bacterial

adhesion among the four groups of stainless steel arch wire, and among the four groups of nickel titanium arch wires respectively, which showed highly significant difference in bacterial adhesion among the four groups of stainless steel and among the four groups of nickel titanium arch wires.

Table 4 Comparison of the number of adherent bacteria MS among four groups of stainless steel and NiTi arch wires separately

Group		15 minutes				60 minutes			
		Mean	Std. Deviation	Kruskal-Wallis test	p-value	Mean	Std. Deviation	Kruskal-Wallis test	p-value
A, B, C and D	Without Saliva	16.41	5.631	61.153	0.000**	71.55	7.679	59.259	0.000**
	With Saliva	10.61	3.278	43.36	0.000**	56.4	7.902	57.161	0.000**
E, F, G and H	Without Saliva	19.85	6.675	58.716	0.000**	73.08	7.733	57.28	0.000**
	With Saliva	15.7	5.795	60.28	0.000**	57.8	12.794	60.619	0.000**

DISCUSSION

The cariogenic *mutans streptococci* colonization have the key roles in occurrence of enamel demineralization associated to orthodontic materials, as these materials in the oral cavity exhibit an exceptional surface that act together beside bacteria, causing pathogenic plaque development for enamel demineralization [1].

The finding of this study proved that there was highly significant difference in bacterial adhesion between the two conditions (with and without saliva) at each duration 15 and 60 minutes for each group A, B, C, D, E, F, G, and H separately (Table 1). This may be due to the development of an early salivary pellicle that will decrease the bacterial adhesion to the arch wires on the contrary to the non-saliva covered arch wires [15-17], these results agree with Burscaa, et al. [18], Papaioannou, et al. [14], Yang, et al. [12], and Al-Lami [19], who reported that the existence of an initial salivary pellicle decrease the number of adhering *mutans streptococci* to orthodontic brackets. These findings disagree with Ahn, et al. [3,20], who found that the saliva covering did not significantly affect the adhesion of bacteria to the orthodontic brackets. The reason of decreasing the amount of bacterial adhesion in the presence of salivary pellicle could be due to the ability of salivary coating to reduce the surface free energy of the underlying materials, in addition to the manifestation of histamines, lysozymes and lactoperoxidase constituents of saliva, that have a unique antimicrobial activities, which could contribute to the impaired adhesion of *mutans streptococci* to saliva treated arch wires *in vitro* [21-23]. In the absence of salivary pellicle-coated arch wires condition, the number of adherent bacteria to all kinds of arch wires in different incubation times was increased. When no conditioning film of saliva was present, the bacterial adhesion was established on the roughness and highly hydrophobic surface, the same situation was noticed by Papaioannou, et al. [14] who reported an encouraged adhesion of *mutans streptococci* in a non-conditioning film of saliva.

Concerning the influence of incubation time, the bacterial colonies adhesion in the covered and non-covered salivary pellicle groups was proportional to the prolonged incubation time and was highest after 60 minutes incubation period than 15 minutes (Table 1), this could agree with Ahn, et al. [3,20] who found that prolonged incubation time encourage the adherence of cariogenic *mutans streptococci*.

Regarding the comparison of the number of bacterial colonies adhesion between different gauges with the same cross section of stainless steel and NiTi arch wires (Table 2), the Mann-Whitney U test showed that there was non-significant statistical difference in bacterial adhesion, except between these groups A and B without saliva at 15 minutes and G and H with saliva at 60 minutes showed significant difference. So, the gauge of the same cross section wires has no effect on the bacterial colonies adhesion, this may be due to the slight difference in thickness of the two groups of arch wires despite having the same cross section. This finding is first tested in this study. Regarding the comparison of the number of bacterial colonies adhesion between stainless steel and NiTi arch wires (Table 3), the Mann-Whitney U test showed that there is highly significant difference, except between group A and E showed significant difference. These results showed that the NiTi arch wires have the greatest number of bacterial colony adhesion, because NiTi arch wire regarded as the roughest wire [20-21]. While the stainless-steel arch wires have the least number of bacterial colony adhesion, due to that the stainless-steel arch wires had the smoothest surface [24-26].

Concerning the comparison of the number of bacterial colonies adhesion among the four groups of stainless steel and NiTi arch separately (Table 4), the result of Kruskal-Wallis test showed that there is a highly significant differences with the bacterial adhesion on rectangular cross section arch wire was more than those with round cross section, which may be due to the larger surface area of the rectangular cross section arch wire than the round ones. This finding is first tested in this study.

No previous studies concerning the relation between bacterial adhesion and arch wires gauge and cross section, yet this was the goal of the present study.

CONCLUSION

So, we can conclude that prolong treatment time promote the adherence of cariogenic *mutans streptococci* and the presence of saliva decreased the number of adherent bacteria, however, the number of adherent bacteria on NiTi arch wire was more than stainless steel ones. Moreover, the amount of bacterial adhesion on rectangular arch wire was more than round arch wire. So, in such conditions, fixed orthodontic patients must increase the frequencies of dental brushing and mouth washing to decrease chances of adherence and numbers of cariogenic *mutans streptococci* colonies.

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