THE EFFECTS OF NITI AND STAINLESS-STEEL ARCHWIRES ON THE ADHERENCE LEVELS OF STREPTOCOCCUS MUTANS

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Abstract

Objectives: Orthodontic appliances remain in the mouth for several months, increasing the possibility of bacterial colonization, especially by *Streptococcus mutans*, a natural oral flora. This study aimed to investigate the adhesion levels of *S. mutans* on nickel-titanium (NiTi) and stainless-steel archwires of different dimensions.

Methods: We performed an *in-vitro* study with two different dimensions of round and rectangular NiTi (40 samples) and stainless-steel (40 samples) archwires. The control group consisted of 8 samples. After infection with *S. mutans*, the archwires were washed with normal saline and cultured in plates containing blood agar media. The numbers of colonies were counted after 48 hours and the colony forming units (CFUs) calculated. Statistical analyses were performed; the Mann-Whitney U test for the comparison between NiTi and stainless-steel wires and the Kruskal-Wallis test for the comparison between wires of different shapes and dimensions.

Results: Adhesion of *S. mutans* on stainless-steel wires was significantly higher ($p<0.000$) than on NiTi orthodontic wires. Moreover, irrespective of the material, rectangular archwires had lower *S. mutans* adhesion in comparison to round archwires, while there was no bacterial growth in the negative control group.

Conclusion: The use of rectangular-shaped NiTi wires in orthodontic treatments was recommended. Adhesion studies with different types of microbes are required to compare the efficacy of various archwires in clinical settings since that would be more accurate and practical than the study of only one type of microbe.

Keywords: Orthodontic Wires, corrosion, nickel, titanium, agar

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Introduction

The demand for orthodontic treatments for the improvement in occlusion and aesthetics has increased significantly. The purpose of using orthodontic treatments is to move the teeth based on a desired pattern until the specific position is achieved (1). However, the use of orthodontic appliances, such as elastic bands, brackets, and archwires, causes oral health problems by interfering with the normal cleaning functions of the mouth and leading to the accumulation of microbial plaque, resulting in tooth decay and periodontal problems (2). Orthodontic devices can alter the balance of microbial ecosystems in the oral cavity by interfering with the food accumulation and adherence surfaces for microbes causing conversion of non-pathogenic bacteria to pathogenic ones (3). One such carcinogenic bacterium is *Streptococcus mutans*, gram-positive cocci, which causes dental plaques due to sucrose fermentation and lactic acid production on tenth enamel (4). The use of orthodontic appliances has escalated the burden of *S. mutans* infection and dental biofilms, increasing the risk of dental caries (2). Bacterial adhesion is influenced by the surface characteristics of biomaterials, particularly surface roughness and surface free energy (SFE). As per thermodynamic rules, a material with a high SFE attracts more bacteria to its surface than the lower counterpart. Increased bacterial contamination and adhesion results in occurrence of tooth decay, particle formation, and gingivitis (5). An archwire is a fixed orthodontic appliance, which significantly increases microbial plaque accumulation (6). Thus, the *S. mutans* adhesion and microbial plaque accumulation on different orthodontic wires for less microbial plaque adhesion have been conducted and improved adhesion was observed (7). Currently, fluoride-based mouthwashes are recommended to remove and reduce the accumulation of plaque around the orthodontic archwires (8). Noncompliance results in increased colonization and accumulation of *S. mutans* and organic products around the wires, thereby lowering the pH, causing tooth decay, periodontal disease, and tooth loss (9). Brackets, bands, and wires in the oral cavity are constantly exposed to changes in the acidity of the mouth due to nutrition, temperature, mechanical fatigue of the wires, and alloy's reaction to corrosion resulting in microbial adhesion (10). Various metal alloys and wires, such as titanium-niobium, thimolium-titanium, super-cables, Bio_Force, Optiflex, and composite wires, along with Nickel-titanium and stainless steel alloys are widely used in orthodontic treatments (1). The later display excellent mechanical and physical properties against corrosion inside the mouth (11).

Titanium wires were first introduced in 1959, are relatively soft, flexible, and have 70% less stiffness as compared to stainless steel archwires resulting in higher formability, making them the most commonly used wires in the initial stages of orthodontics (1). However, Stainless steel wires used in the manufacturing of ligature and archwires (1). Specific attention must be paid to microbial adhesion and aggregation using these two types of wires to maintain oral hygiene (12). Clarke first reported the distribution and adhesion of microbial plaque on orthodontic appliances, and the importance of microbial plaque control (13). A few studies on *S. mutans* plaque adhesion on different types of orthodontic wires, show contradictory results (14-16). Thus, we evaluated the *S. mutans in vitro* adhesion as well as its accumulation on orthodontic wires of varying diameters.

METHODS

We conducted a randomized *in vitro* study with 80 samples, split into two 40-unit-groups, in addition to an eight-sample negative control group. Also, the rights of the human or animal subjects were protected, and the approval was obtained from a known identified institutional review board, and it was based on the thesis which was reported in the acknowledgement of this article.

The sample size was estimated from previous studies, with a minimum of 10 and a maximum of 48 in each subgroup (17,18). The archwires were manufactured by American Orthodontics and composed of nickel-titanium (NiTi) and stainless steel. One group was assigned NiTi and the other stainless-steel wires. In addition, the wires in both the groups were of different cross-sections and dimensions, round (0.016-inch, 0.018-inch) and rectangular (0.016 × 0.022-inch, 0.017 × 0.025-inch); thus, each group had 10 units of each size (Figure 1).

Initially, 5 cm of each archwire was cut randomly from the posterior and anterior portions. Ten of each type of the wires was sterilized by autoclave. To measure the turbidity of a pure *S.* (ATCC35668) *mutans*, 0.5 Macfarland (1.5×10^{8}CFU) suspension was prepared (19). For germ replication, a suspension with eight pure *S. mutans* at a fixed volume was prepared in one Erlenmeyer flask for each subgroup of archwires. Each subgroup was immersed in a *S. mutans* suspension and incubated for 2 h at 37°C (19). Next, each type of wire was removed by using sterile pence, the later were heat sterilized on a flame and removed post cooling. Lastly, the wires were washed with sterile saline for 2 min to remove any remaining *S. mutans*. Before pouring into the plates, 5% sterile Mammalian blood was added after autoclaving. The specimens were plated on blood agar media in such a way that one surface of the wire was first sampled by a swab and cultured in the medium, while the other surfaces were placed directly on gel medium (20). Two negative and positive control groups were identified for the sake of accuracy. In the negative control group, the non-incubated wires were placed on the blood agar medium. The positive control group only included *S. mutans* colonies (Figure 2).

The bacterial media (peptone 10 gram/lit, tryptose 10 g/lit, sodium chloride 5g/lit, agar 15 g/lit) was incubated for 48 h at 37°C, and the number of *S. mutans* colonies were counted visually and represented as colony forming units (CFUs) (Figure 3). Statistical analyses included Mann-Whitney U and Kruskal-Wallis test to compare the NiTi and stainless-steel wire groups between the eight subgroups. This research was performed in the Microbiology Laboratory of the Dental School of Islamic Azad University.

RESULTS

The amount of CFU according to the type of alloy is presented in Table 1 and shows that it was 48.10 in the steel group and 82.6 in the nickel-titanium group and the statistical test showed that the difference was statistically significant ($p < 0.0000$).

The amount of CFU based on the type of alloy and their diameter is presented in Table 2 and shows that in steel group:

Firstly, in round cross section, the amount of adhesion is more than rectangular one. As an example, the amount of adhesion to the 0.018 round wires was equal to 13.24 and in rectangular section (0.016 × 0.022) was equal to 8.78 which was 33.6% less and this was statistically significant ($p < 0.0000$).

Secondly, in the case of nickel-titanium with a round cross section, the degree of adhesion is higher than the rectangular one. For instance, in 0.018 cross section, the adhesion rate was 8.62 and in 0.017 × 0.025. It was equal to 5.78 that was 33% less and this difference was statistically significant ($p < 0.0000$).

In the third place, which of the two alloys has more adhesion, regardless of their cross sectional shapes (round or rectangular). It shows that the degree of adhesion of steel wire is always higher than nickel-titanium. As a case in point, in cross section 16, steel wires had a cross section of 10.28 and in nickel titanium it was 7.42 with a difference of 27.8% less (P <O.000). This was true for other cross sections as well.

Meanwhile, ANOVA test showed the difference between these 8 groups that was statistically significant ($P < 0.000$). From another view, SCHEFF post hoc test demonstrated that the lowest adhesion was related to 0.016 ×0.022 NiTi with 5.48 and the most amount of adhesion was related to round steel wires with a cross section of 0.018 and it was 13.24 ($P < 0.0000$) (Table 2).

The degree of adhesion of Streptococcus mutans, according to the type of alloy and cross section, (by adding the cross sections of 0.016 and 0.018 wires) is presented in Table 3.

As it is clear and in the first place, the adhesion was 11.76 when it was round and 9.2 in the rectangular cross section wires, which was 23% less than the round cross section ($p < 0.0000$).

Secondly, in the case of nickel-titanium alloy, when the cross section was round, the adhesion was 8.02 and with rectangular cross section was 5.63, which was 30% less ($P < 0.0000$).

Discussion

The results of this study showed that the adhesion of *S. mutans* on stainless-steel wires was significantly higher than on NiTi orthodontic wires. Moreover, adhesion was significantly less on the rectangular wires compared to round wires.

The orthodontic archwires have been fabricated from several alloys containing different concentrations of nickel, cobalt, and chromium. Biodegradation of the alloys ideally occurs in the oral cavity due to temperature and microbes-enzymes reaction. Physiological stress can also change salivary inorganic trace element content, which might affect the health of teeth, enamel, and oral mucosal tissues (21). Metals like nickel and chromium are known to be cytotoxic, mutagenic, and allergic (22). Nickel also causes a higher rate of allergy than these other metals (1).

Although NiTi is the most important wire used in the early stages of orthodontic treatment and has good mechanical performances with excellent properties, these wires contain more than 50% nickel. In comparison, stainless-steel wires only contain 8% nickel, although some studies have shown the existence of nickel (1,23). Both wires are associated with the release of ions, not related to the metal percentage composition of the wire (24). Metal ions have a higher chance of release from stainless-steel wires composed of copper, chromium, iron, and nickel. In contrast, only nickel and titanium are released from NiTi wires (25). Van Dijk *et al* (26). showed that the surface energy of any material could affect microorganism colonization; thus, the higher the energy, the greater the colonization. Therefore, higher ion release and more surface energy lead to greater microbial adhesion. In agreement, the current study also showed higher microbial adhesion on stainless steel wires.

Another aspect of orthodontic metal/alloy exposure to oral fluids is galvanic corrosion, occurring due to the differential metal corrosion potential when in electrical contact (24). Corrosion causes surface roughness, forms pores, and releases ions from the metal/alloy leading to an increase in microbial adhesion. Surface roughness on orthodontic wires can affect corrosion, friction, tooth movement, and tissue compatibility (27). Titanium wires are soft and flexible, with 70% lower stiffness compared to stainless-steel wires (1). NiTi wires have lower load-deflection but a greater range of activity (28). Therefore, a reduction in the hardness and stiffness of titanium wires can prevent microbial adhesion. Shin *et al* (29). immersed steel and NiTi archwires into synthetic saliva and observed surface corrosion after three months via electron microscopy. They observed corrosion in the stainless-steel wires but a limited color change in the NiTi wires. The amount of corrosion increased over time, leading to weakening and breaking of the stainless-steel wires (29). A similar observation was recorded in our study, where the NiTi wires could resist corrosion, while the stainless-steel wires could not. Yo *et al* (30). demonstrated that, although stainless-steel wires have lower surface roughness, NiTi alloys have a more regular surface, leading to lower friction and lesser microbial adhesion.

Increased microbial adhesion to round wires could be due to the uniform surface of the round wire as well as the dextraze enzyme and glycogen from *S. mutans* (31). Recently, adhesion of the novel coronavirus (COVID-19) was found to be higher on smooth and varnished surfaces than on unvarnished surfaces (32).

According to the data obtained from the manufacturer, 0.016-inchand 0.016 × 0.022-inchwires are less thick and more flexible than 0.018-inchand 0.017 × 0.025-inchwires, respectively, irrespective of the material. Our study showed that microbial adhesion decreases with an increase in the size of the wire and that the greater porosity of small wires leads to a greater amount of microbe replacement. In addition, wires with different diameters exert a different amount of force and can have a non-uniform performance under the same conditions.

The results showed significantly increased adhesion of *S. mutans* on round wires than on rectangular wires. One of the reasons could be the rate of metal ion release. A study by Azizi et al. evaluated metal ions release from NiTi rectangular archwires of similar sizes after 1 h, 24 h, 1 week, and 3 weeks, showing that the rate of ion release was different depending on the surface and shape of the wires. The amount of nickel released from the rectangular wires was higher than the round wires (33). Our study showed a contrasting result as adhesion was higher on round wires. We speculated that this was due to the rate of ion release and the size of the wires considered in this study. Therefore, we postulated that size has a greater impact than the shape of the wires.

This study had several limitations. Quantitative measurement of microbial adhesion to the brackets could not be done due to the lack of an electronic microscope. Our results reflect an *in vitro* environment, where the microbe was grown in a laboratory, which can be different from an *in vivo* experiment. We tested the adhesion level of only *S. mutans* and did not address its rate of adhesion on other types of wires, like beta titanium wires. Additionally, since we had no access to SEM, we used the CFU Count Method to count the colonies.

Despite the study limitations, there were several strengths to this research. First, we were not supported or biased by anyone or any manufacturer. Additionally, we considered two types of wires with two different cross-sections. Moreover, our statistical analyses involved non-parametric tests, whereas other studies have used parametric tests, like T-test or ANOVA which are not accurate.

CONCLUSION

The amount of *S. mutans* adhesion depends on the material, shape, and size of the orthodontic wires. The roughness of the surface determined the extent of microbial adhesion. Therefore, orthodontic wires must be made of metal alloys with greater corrosion resistance. Hence, we recommend the use of rectangular-shaped NiTi wires in orthodontic treatments. Adhesion studies with different types of microbes are required to compare the efficacy of various archwires in clinical settings since that would be more accurate and practical than the study of only one type of microbe. A more comprehensive study using a scanning electron microscope might yield better results for clinical use. In future studies, identifying and investigating several factors affecting the adhesion of *S. mutans*, such as lactobacilli, staphylococci, *Veillonella*, and yeast, may provide a better perspective related to orthodontic promotion. Finally, ion release from commercial wires and brackets, the corrosion of wires, and microbial adhesion to wires requires more sophisticated studies and should be considered by different manufacturers.

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Conflict of interest

The authors have no competing interests to declare.

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Table 1 The rate of *Streptococcus mutans* adhesion based on the wire material.

Streptococcus mutans adhesion Materials	Mean ± SD (CFU)	P Value
Stainless-steel	$10.48 \times 10^3 \pm 2.05$	0.000*
NiTi	$6.82 \times 10^3 \pm 1.54$	0.000

*Statistically significant

Figure Legends:

Figure 1. An example of a nickel-titanium and stainless-steel wire of 0.017×0.025-inch diameter.

Figure 2. Positive and negative control groups.

Figure 3. *Streptococcus mutans* colony growth on blood agar media.