



Comparison of *Streptococcus mutans* Adhesion to Fixed Orthodontic Wires in different Types of Saliva under Laboratory Conditions

Kamal Mirmohammadi¹ and Ahmad SheibaniNia^{1,*}

¹Department of Orthodontics, TeMS.C., Islamic Azad University, Tehran, Iran

Abstract:

Introduction: This study investigates the adhesion of *Streptococcus mutans* to fixed orthodontic wires in varying types of saliva under laboratory conditions, given the heightened microbial load and increased caries prevalence in patients with orthodontic appliances.

Materials and Methods: Stainless steel and nickel-titanium arch-wires of the same dimensions were utilized. In this study, a total of 24 samples in six sub group of were used. After preparation and sterilization, the samples were immersed in artificial saliva solutions for two hours with either normal or acidic pH. Subsequently, the samples were incubated in an *S. mutans* suspension. Following serial dilution and cultivation on brain heart infusion (BHI) agar, the number of former colonies was counted. The data were analyzed using two-way ANOVA with a significance level of $p \leq 0.05$.

Results: The adhesion rate of *S. mutans* to the stainless steel arch-wires was significantly higher than the nickel-titanium arch-wires in both acidic and normal saliva ($p \leq 0.05$). Moreover, the adhesion rate of both arch-wires was lower in acidic saliva compared to normal saliva ($p \leq 0.05$).

Discussion: According to the results of this research, the adhesion rates of *S. mutans* were different for arch-wire materials. This research provided insights that could guide the selection of materials used in orthodontics.

Conclusion: *S. mutans* exhibits a lower adhesion rate of the nickel-titanium arch-wires compared to the stainless steel arch-wires in both acidic and normal saliva.

Keywords: Acidic saliva, Orthodontic treatment, Orthodontic wires, Nickel-titanium arch-wires, Saliva, Stainless steel arch-wires, *Streptococcus mutans*.

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*Address correspondence to this author at the Department of Orthodontics, Tehran Medical Sciences, Islamic Azad University, Tehran, Iran; E-mails: asheibaninia@yahoo.com or Sheibania@iau.ac.ir

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1. INTRODUCTION

Modern orthodontic treatments encompass a series of orthodontic and orthognathic interventions aimed at aligning the soft tissue with the position of the teeth and achieving functional occlusion. These procedures are commonly performed by orthodontists to adjust the position of the teeth or the jaw, thereby enhancing soft tissue relationships and creating functional occlusion [1,

2]. Moreover, the importance of frequent dental examinations during orthodontic treatment is well recognized among orthodontists, as investigations have shown that patients undergoing fixed orthodontic treatment are at a higher risk for caries and periodontal problems in comparison to others [3]. Results of investigations indicate that 96% of orthodontic patients develop at least one white lesion during treatment,

predominantly on the buccal surface of the upper anterior teeth near the brackets [4]. It has also been observed that patients with a high risk of caries before treatment tend to have higher levels of *Streptococcus mutans* in their saliva during treatment [1, 2]. This is attributed to the rapid alteration of oral flora, favoring pathogenic microorganisms after the commencement of treatment with fixed appliances and a decrease in salivary pH three months into treatment, contributing to the acidification of the oral environment and the appearance of white lesions [5-7].

The utilization of intraoral appliances, such as arch-wires and other orthodontic tools, increases the accumulation of caries-causing microorganisms by expanding contact points and limiting toothbrush access to all dental surfaces. In general, the use of fixed intraoral appliances increases the available surfaces for microorganism attachment, thereby enhancing the potential for biofilm formation [8].

A crucial study comparing the amount of *S. mutans* in orthodontic patients with active caries to those without dental caries revealed that the number of *S. mutans* in orthodontic patients is influenced by the number of teeth with active caries [9]. Orthodontic treatment is thus a risk factor for periodontal diseases and caries, with an increase in cariogenic microorganisms like *S. mutans*, leading to enamel and dentin demineralization and eventual caries in this patient group [10-12].

Controlling plaque indicators in patients before treatment is essential. Therefore, understanding the degree of *S. mutans* adhesion to commonly used orthodontic arch-wires, such as stainless steel and nickel-titanium, is crucial for selecting appropriate orthodontic arch-wires based on the type of saliva. A review of previous studies reveals a lack of sufficient information on the extent of *S. mutans* adhesion to fixed orthodontic materials and devices, indicating a knowledge gap in this area. Therefore, the primary outcome of this research was to compare the extent of *S. mutans* adhesion to fixed orthodontic arch-wires in different types of saliva under laboratory conditions.

2. MATERIALS AND METHODS

This *in vitro* study investigated the adhesion of *S. mutans* to stainless steel and nickel-titanium arch-wires, both measuring 0.016 x 0.022 inches, was investigated. According to do Rosário Junior [13] the required sample size was calculated using the fixed effects ANOVA analysis with $\alpha = 0.05$ and $\beta = 0.2$ for the material type variable and $\beta = 0.01$ for the saliva type variable. The effect size was 0.6 for the material type and 1.5 for the saliva type. A total of 24 samples in six subgroups were evaluated.

S. mutans UA159 (ATCC 700610) was obtained from the National Center for Genetic and Biological Resources of Iran. The bacteria were cultured in brain heart infusion (BHI) with 100 mM glucose at 37 °C under 10% CO₂. After 24-48 hours, bacterial cells were centrifuged at 3,000 g, washed twice with sterile 145 mM NaCl, and re-suspended

to a concentration of 1.5×10^8 CFU/mL, confirmed spectrophotometrically at 620 nm (Varian Cary 50 UV-Vis, Varian Inc., Mulgrave, VIC, Australia) [13].

Normal artificial saliva was prepared [14, 15] from the Biology Center of Tehran Medical Sciences, Islamic Azad University, Iran. Two samples of artificial saliva with pH equal to 5 and 7 were prepared. To prepare 1 liter of artificial saliva, dissolve 0.4 g of sodium chloride (NaCl) and 0.4 g of potassium chloride (KCl) in approximately 800 mL of distilled water to provide the primary Na⁺, K⁺, and Cl⁻ ions found in natural saliva. Next, add 0.795 g calcium chloride dihydrate (CaCl₂·2H₂O) and 0.78 g disodium hydrogen phosphate dihydrate (Na₂HPO₄·2H₂O) to provide Ca²⁺ and phosphate buffering capacity, then stir until all salts are fully dissolved. To mimic the antimicrobial thiocyanate, stir in 0.005 g potassium thiocyanate (KSCN) along with 0.005 g sodium sulfide nonahydrate (Na₂S·9H₂O) for trace sulfide content. Add 1 g urea as a nitrogenous component. Gradually sprinkle in 2.5 g of 1% carboxymethylcellulose (CMC) under continuous magnetic stirring to achieve a final viscosity similar to natural saliva (0.25-1% CMC may be used depending on desired thickness). Then, incorporate 5 mL glycerin as a humectant to enhance moisture retention and mouth feel. Adjust the pH of the mixture to 6.8-7.2 using dilute NaOH or HCl. Finally, bring the total volume to 1 L with distilled water, mix thoroughly, and, if long-term storage is required, sterilize by passing through a 0.22 µm filter and store at 4 °C in sterile bottles [14, 15]. Phosphate-buffered saline with pH 7.2 served as the control group [16]. Artificial saliva for replicating natural saliva's composition was procured from Arad Company, Tehran, Iran.

In this investigation, two types of arch-wires (stainless steel and nickel-titanium) (American Orthodontics, USA) were utilized. Each subgroup was immersed in artificial saliva for 2 hours to facilitate pellicle formation, and then incubated at 37 °C and 5% CO₂ in a *S. mutans* suspension [13, 17]. The arch-wires (10 mm long) were disinfected using an ultrasonic device (Mini Sono Cleaner CA 1470, Kaijo Denki Co. Ltd., Tokyo, Japan) for 15 min, followed by immersion in 70% alcohol for 30 min. Sterility was confirmed using BHI culture medium for 24 hours [17]. Post-incubation, the samples were washed thrice with 500 µL of 0.9% saline to remove unattached bacteria. The samples were then sonicated at 50% strength (Qsonica 125, Newtown, CT, USA) in 10 mL of 0.9% saline for 3 seconds. The resulting suspensions were serially diluted, and 10 µL of each dilution was cultured on BHI agar using the dropwise method, in six replicates. The culture plates were incubated at 37 ± 1 °C for 48 hours, after which colony-forming units (CFUs) were counted using the pour plate method [18].

Data collected from our research were analyzed using Microsoft Excel and Statistical Package for the Social Sciences (SPSS, version 21, Armonk, New York, USA). The data were analyzed using two-way ANOVA with a significance level of $p \leq 0.05$. The significance level was set at $P < 0.05$. Descriptive statistics, including means and

standard deviations (\pm SD) and standard error (\pm SE), were calculated for the adhesion rates at pH levels of 5 and 7.

3. RESULTS

The effect of experimental factors and artificial saliva pH on the amount of *S. mutans* bacteria indicated that the factors of orthodontic wire type and pH had a significant impact on *S. mutans* colonies (Table 1). The *S. mutans* colonies formed in saliva with a pH equal to 5 in the stainless steel arch-wires (61,650) were 5.2 times more than the group of nickel titanium arch-wires (7,325) (Table 1). The number of *S. mutans* colonies formed in saliva with a pH of 7 in the group of the stainless steel arch-wires was 2.57 times more than the nickel titanium arch-wires ($p \leq 0.05$).

Table 1. Descriptive results of the number of *S. mutans* colonies formed on each of the orthodontic wires in all types of saliva.

pH	Material	Number of Bacterial Colonies \pm SD (CFU/mL)	P-value
5	SS	177526 \pm 61650	0.000
	NiTi	33896 \pm 7325	
7	SS	1104056 \pm 110435	0.000
	NiTi	429280 \pm 96453	

Table 2. Comparison of the amount of *S. mutans* bacteria in the studied groups in acidic and normal saliva.

Material	pH	Mean	Std. Deviation	Std. Error Mean	P-value
SS	7	1104056.66	110435.55	45085.12	0.000
	5	177526.66	61650.29	25168.63	
NiTi	7	429280	96453.49	39376.98	0.000
	5	33896.66	7325.64	2990.68	

According to the results shown in Table 2, there is a significant difference between the amounts of bacteria at pH 5 and 7 in all groups. The number of bacteria attached to the nickel-titanium arch-wires at pH 7 is more than 5 ($p \leq 0.05$). The number of bacteria attached to the stainless steel arch-wires at pH 7 is more than at pH 5 ($p \leq 0.05$).

4. DISCUSSION

The present study demonstrated the significant role of the metallic construction of the arch-wires in the colonization of microorganisms. Specifically, it was found that *S. mutans* attachment was lower on the nickel-titanium arch-wires compared to the stainless steel arch-wires in both normal and acidic saliva conditions. A key contributing factor is the greater release of metal ions from the nickel-titanium arch-wires, which imparts antibacterial properties [19]. Hepyükselen *et al.* [20] noted that the nickel-titanium arch-wires, particularly those coated with other metals such as copper, exhibited

enhanced the (delete "the") antibacterial properties, reducing the likelihood of colonization by *Streptococcus* and *Lactobacillus* species. Eldriny *et al.* [21] reported higher adhesion rates on stainless steel arch-wires, which significantly decreased following the use of a natural antibacterial mouthwash. Polke *et al.* [22] found that biofilm accumulation was significant across all arch-wire types, with the highest accumulation on the titanium-molybdenum alloy (TMA) samples and the least on the coated stainless steel arch-wire samples.

Our findings revealed a higher bacterial colonization in normal saliva compared to acidic saliva. Similarly, Laird *et al.* [23] reported that low pH conditions increase the release of metal ions from arch-wires, enhancing their antibacterial properties and reducing *S. mutans* attachment. However, recent studies have not examined different incubation times when highlighting the influence of saliva pH on bacterial attachment; they did report increased bacterial binding in normal saliva compared to acidic conditions.

S. mutans is known for its acidogenic properties, producing acidic compounds that inhibit the growth and attachment of certain streptococcal species. The prevalence of *S. mutans* has been a focal point of research for patients undergoing orthodontic treatment [3, 24]. This comprehensive laboratory study compared two widely used types of arch-wires, *i.e.*, stainless steel and nickel-titanium, under normal and acidic saliva conditions. The results indicate that both the type of arch-wire and the salivary pH significantly affect the binding rate of bacteria. Saliva and the formation of a salivary pellicle on orthodontic appliances reduce *S. mutans* binding [12]. This reduction is partly due to the presence of lysozyme enzymes, histatins, and antibacterial elements in saliva, but mainly due to salivary pellicles. Certain salivary proteins, such as cystatins, amylase, immunoglobulin A, and mucin-7 (MG2), have a high affinity for binding to bracket surfaces and simultaneously possess active receptors for microorganisms. Ahn *et al.* [25] found that *Streptococcus gordonii* has a high affinity for binding to MG2 salivary pellicles and amylases, whereas *S. mutans* does not, as these pellicles do not provide suitable receptors for it. Two other critical factors influencing the attachment of microorganisms are surface free energy and surface topography. A saliva coating reduces the surface energy, thereby decreasing the probability of bacterial attachment [26].

Generally, understanding the factors that influence the adhesion of *S. mutans*, as a key pathogen in dental caries, is crucial for improving patient outcomes. Moreover, the composition of saliva, especially its pH and buffering capacity, plays an important role in oral health and the prevention of caries. By investigating the adhesion rates of *S. mutans* to different arch-wire materials in controlled laboratory conditions, this study provides insights that could guide the selection of materials utilized in orthodontics.

CONCLUSION

The presence of orthodontic appliances alters the oral environment, fostering the growth and accumulation of cariogenic microorganisms. This study revealed that stainless steel arch-wires significantly enhanced *S. mutans* colonization, thereby elevating the risk of dental caries and white lesions. One of the strengths of this study was its use of two types of saliva to simulate normal and carious conditions. In general, a limitation of this study was the lack of clinical conditions and other influencing factors in salivary caries. This laboratory study can help in making informed decisions about treatment plans and the selection of materials and devices for patients at high risk of caries, laying the groundwork for future clinical studies. Based on the results of this study, it is recommended to use nickel-titanium arch-wires whenever possible in patients with a high risk of caries.

AUTHORS' CONTRIBUTIONS

The authors confirm contribution to the article as follows: A.S.: Study conception and design; K.M.: Data collection; A.S. and K.M.: Analysis and interpretation of results; K. M.: Draft manuscript. All authors reviewed the results and approved the final version of the manuscript.

LIST OF ABBREVIATIONS

SPSS = Statistical Package for the Social Sciences

TMA = Titanium-Molybdenum Alloy

ANOVA = Analysis of Variance

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Not applicable.

HUMAN AND ANIMAL RIGHTS

Not applicable.

CONSENT FOR PUBLICATION

Not applicable .

AVAILABILITY OF DATA AND MATERIALS

The data supporting the findings of this study are presented within the article.

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CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

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REFERENCES

- [1] Mirmohammadi K, Sheibani Nia A, Safarzadeh Khosroshahi S, Shokouhi Mostafavi SK. *In vitro* comparison of *Streptococcus mutans* adhesion to orthodontic composites in various types of saliva. *J Res Dent Sci* 2024; 21(4): 281-91.

- [2] Belviridy LM, Sheibaninia A, Khosroshahi SS, Valinejadi A, Mostafavi SKS, Fard MJK. Influence of bracket and composite material in conjunction with saliva pH on *Streptococcus mutans* adhesion: A controlled *In vitro* investigation. *Biomed Biotechnol Res J* 2025; 9(2): 119-24.
http://dx.doi.org/10.4103/bbrj.bbrj_187_25
- [3] Dale CLT, Smorthit K, Storey M, Srinivasan V. The importance of the Basic Periodontal Examination for paediatric orthodontic patients. *Br Dent J* 2021; 231(3): 163-8.
<http://dx.doi.org/10.1038/s41415-021-3292-5> PMID: 34385643
- [4] Almutairi RM, Alturaif DJ, Alanzi LM. Importance of oral hygiene in orthodontic treatment. *Saudi J Oral Dent Res* 2023; 8(3): 100-9.
<http://dx.doi.org/10.36348/sjodr.2023.v08i03.001>
- [5] Das A. Salivary *Streptococcus mutans* level in patients before, during and after fixed orthodontic treatment in Bengalee population. *J Adv Med Dent Sci Res* 2024; 12(11): 51-5.
<http://dx.doi.org/10.21276/jamdsr>
- [6] Cardoso AA, Lopes LM, Rodrigues LP, Teixeira JJ, Steiner-Oliveira C, Nobre-dos-Santos M. Influence of salivary parameters in the caries development in orthodontic patients—An observational clinical study. *Int J Paediatr Dent* 2017; 27(6): 540-50.
<http://dx.doi.org/10.1111/ipd.12295> PMID: 28247450
- [7] Sheibaninia A, Sepasi S, Saghiri MA, Sepasi S. The effect of an acidic food-simulating environment on the shear bond strength of self-ligating brackets with different base designs. *Int J Dent* 2014; 2014(1): 1-5.
<http://dx.doi.org/10.1155/2014/689536> PMID: 25328524
- [8] Lucchese A, Bondemark L, Marcolina M, Manuelli M. Changes in oral microbiota due to orthodontic appliances: A systematic review. *J Oral Microbiol* 2018; 10(1): 1476645.
<http://dx.doi.org/10.1080/20002297.2018.1476645> PMID: 29988826
- [9] Lim BS, Kim BH, Shon WJ, Ahn SJ. Effects of caries activity on compositions of mutans streptococci in Saliva-Induced biofilm formed on bracket materials. *Materials* 2020; 13(21): 4764.
<http://dx.doi.org/10.3390/ma13214764> PMID: 33114489
- [10] Contaldo M, Lucchese A, Lajolo C, et al. The oral microbiota changes in orthodontic patients and effects on oral health: An overview. *J Clin Med* 2021; 10(4): 780.
<http://dx.doi.org/10.3390/jcm10040780> PMID: 33669186
- [11] da Costa Rosa T, de Almeida Neves A, Azcarate-Peril MA, et al. The bacterial microbiome and metabolome in caries progression and arrest. *J Oral Microbiol* 2021; 13(1): 1886748.
<http://dx.doi.org/10.1080/20002297.2021.1886748> PMID: 34188775
- [12] Belviridy LM, Sheibaninia A, Khosroshahi SS, Mostafavi SKS, Fard MJK. Comparison of *Streptococcus mutans* adhesion with different concentrations in artificial saliva to fixed orthodontic materials in laboratory conditions. *Biomed Biotechnol Res J* 2024; 8(4): 428-33.
http://dx.doi.org/10.4103/bbrj.bbrj_276_24
- [13] do Rosário AF, Knop LAH, Baboni FB, Rymovicz AUM, Tanaka OM, Rosa EAR. Differential adhesion of *Streptococcus mutans* to metallic brackets induced by saliva from caries-free and caries-active individuals. *J Investig Clin Dent* 2011; 2(3): 197-200.
<http://dx.doi.org/10.1111/j.2041-1626.2011.00058.x> PMID: 25426791
- [14] Mohammed H, Rizk MZ, Wafaie K, Ulhaq A, Almuzian M. Reminders improve oral hygiene and adherence to appointments in orthodontic patients: A systematic review and meta-analysis. *Eur J Orthod* 2019; 41(2): 204-13.
<http://dx.doi.org/10.1093/ejo/cjy045> PMID: 29947755
- [15] Baines S, Hensels IS, Talmi D. The use of 'artificial saliva' as a neutral control condition in gustatory research. *Physiol Behav* 2021; 229: 113254.
<http://dx.doi.org/10.1016/j.physbeh.2020.113254> PMID: 33220327
- [16] Pytko-Polonczyk J, Jakubik A, Przeklasa-Bierowiec A, Muszynska B. Artificial saliva and its use in biological experiments. *J Physiol Pharmacol* 2017; 68(6): 807-13.

- PMID: 29550792
- [17] Shalchi M, Hajian-Tilaki A, Sadegh Khanjani M, Sabzgol P, Aghajani Nargesi R. Comparing *Streptococcus mutans* adhesion using different orthodontic bracket ligations: An *in vitro* study. *J Dentomaxillofacial Radiol Pathol Surg* 2018; 7: 7-12. <http://dx.doi.org/10.32598/3dj.7.1.7>
- [18] Arango-Santander S, Gonzalez C, Aguilar A, *et al.* Assessment of *Streptococcus mutans* adhesion to the surface of biomimetically-modified orthodontic archwires. *Coatings* 2020; 10(3): 201-10. <http://dx.doi.org/10.3390/coatings10030201>
- [19] Özcan Z, A Y. A narrative review of nickel ion release from NiTi and stainless steel orthodontic archwires: *In vitro* and *in vivo* perspectives. *Int J Dent Res Allied Sci* 2025; 5(2): 125-39. <http://dx.doi.org/10.51847/a1w5WPFNz1>
- [20] Hepyükselen BG, Cesur MG. Comparison of the microbial flora from different orthodontic archwires using a cultivation method and PCR: A prospective study. *Orthod Craniofac Res* 2019; 22(4): 354-60. <http://dx.doi.org/10.1111/ocr.12335> PMID: 31294901
- [21] ELdriny A, Ibrahim S, Abdel Samad F, Ali H. Evaluation of the adhesion of *Streptococcus mutans* to different orthodontic arch wires and the effect of honey mouth wash on it. *Al-Azhar Dent J Girls* 2020; 7(4): 643-8. <http://dx.doi.org/10.21608/adjg.2020.17843.1198>
- [22] Polke P, Jain U, Marothiya S, Agrawal P, Dixit S, Dubey C. Comparative evaluation of biofilm adhesion to different types of archwire and microbiological colonization during orthodontic treatment. *J Indian Orthod Soc* 2021; 55(2): 150-7. <http://dx.doi.org/10.1177/0301574220957794>
- [23] Laird C, Xu X, Yu Q, Armbruster P, Ballard R. Nickel and chromium ion release from coated and uncoated orthodontic archwires under different pH levels and exposure times. *J Oral Biosci/ JAOB, Jpn Assoc Oral Biol* 2021; 63(4): 450-4. <http://dx.doi.org/10.1016/j.job.2021.10.007> PMID: 34740833
- [24] Jakavičė R, Žarovienė A. Changes in the pH and the flow rate of saliva during orthodontic treatment with fixed orthodontic appliances: A systematic review. *Turk J Orthod* 2023; 36(3): 199-207. <http://dx.doi.org/10.4274/TurkJOrthod.2022.2022.66> PMID: 37782023
- [25] Ahn SJ, Kho HS, Lee SW, Nahm DS. Roles of salivary proteins in the adherence of oral streptococci to various orthodontic brackets. *J Dent Res* 2002; 81(6): 411-5. <http://dx.doi.org/10.1177/154405910208100611> PMID: 12097434
- [26] Fatani EJ, Almutairi HH, Alharbi AO, *et al.* *In vitro* assessment of stainless steel orthodontic brackets coated with titanium oxide mixed Ag for anti-adherent and antibacterial properties against *Streptococcus mutans* and *Porphyromonas gingivalis*. *Microb Pathog* 2017; 112: 190-4. <http://dx.doi.org/10.1016/j.micpath.2017.09.052> PMID: 28966064

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