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Evaluation of the Effects of Different Orthodontic Wire Material Compositions and Cross-Sectional Forms on In Vitro *Streptococcus mutans* Adhesion

Farklı Ortodontik Tel Materyalleri Bileşimi ve Kesit Geometrilerinin In Vitro *Streptococcus mutans* Adezyonuna Etkisinin Değerlendirilmesi

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ABSTRACT

Introduction: *The present in vitro study was conducted to evaluate the effects of different orthodontic wire materials and cross-sectional geometries on Streptococcus mutans biofilm formation and bacterial viability.*

Materials and Methods: *The study examined three distinct wire materials: Stainless steel (SS), nickel-titanium (NiTi), and copper containing nickel-titanium (Cu–NiTi). Each material was prepared in two cross-sectional forms: round (0.016″) and rectangular (0.016″ × 0.022″). Twelve samples from each group were incubated with S. mutans ATCC 25175 strain at 37 °C and 5% CO_2 for 24 hours. The measurement of biofilm biomass was conducted by means of crystal violet staining at OD_{570}. The assessment of bacterial viability was conducted through the quantification of colony-forming units (CFU/mL) following a process of vortex-ultrasonication separation. The subsequent analysis of the data was conducted using two-way analysis of variance (Robust analysis of variance) and Holm-corrected post-hoc tests. The level of statistical significance was set at $p< 0.05$.*

Results: *The composition of the wire material and the cross-sectional shape of the wire exhibited a significant impact on the accumulation of biofilm and bacterial adhesion ($p< 0.001$). Rectangular cross-section NiTi wires demonstrated the highest OD_{570} and CFU values, while Cu-NiTi (both cross-sections) and round cross-section SS wires exhibited the lowest bacterial loads ($p< 0.001$). A higher degree of biofilm accumulation was observed in rectangular cross-section wires compared to round cross-section wires ($p< 0.001$).*

Conclusion: *The composition and geometry of the wire material jointly affect bacterial adhesion on orthodontic arch wires. In addition, Cu–NiTi wires demonstrated a reduced tendency for biofilm accumulation, an outcome that can be attributed to the inherent antimicrobial properties of copper. The findings emphasize the significance of material selection and wire design in minimizing microbial colonization during orthodontic treatment.*

Key Words: *Biofilm; Bacterial adhesion; Copper; Orthodontic wires; Streptococcus mutans*

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ÖZ

Farklı Ortodontik Tel Materyalleri Bileşimi ve Kesit Geometrilerinin In Vitro *Streptococcus mutans* Adezyonuna Etkisinin Değerlendirilmesi

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Giriş: *Bu in vitro çalışma, farklı ortodontik tel materyallerinin ve kesit geometrilerinin Streptococcus mutans biyofilm oluşumu ve bakteriyel canlılık üzerindeki etkilerini değerlendirmek amacıyla yapılmıştır.*

Materyal ve Metod: *Çalışmada paslanmaz çelik (SS), nikel-titanyum (NiTi) ve bakır içerikli nikel-titanyum (Cu–NiTi) olmak üzere üç farklı tel materyali incelenmiştir. Her materyal, yuvarlak (0.016") ve dikdörtgen (0.016" × 0.022") olmak üzere iki kesit formunda hazırlanmıştır. Her bir gruptan 12 adet numune, S. mutans ATCC 25175 suşu ile 37 °C ve %5* CO_2 *koşullarında 24 saat inkübe edilmiştir. Biyofilm biyokütlesi,* OD_{570}*'te kristal viyole boyama ile ölçülmüştür. Bakteriyel canlılık ise vorteks-ultrasonikasyon ayrıştırma işleminden sonra koloni oluşturan birim (CFU/mL) sayımı ile değerlendirilmiştir. Veriler iki yönlü varyans analizi (Robust analysis of variance) ve Holm düzeltmeli post-hoc testlerle analiz edilmiştir. İstatistiksel anlamlılık düzeyi p< 0.05 olarak kabul edilmiştir.*

Bulgular: *Hem tel materyali bileşimi hem de kesit formu, biyofilm birikimi ve bakteriyel yapışmayı anlamlı düzeyde etkilemiştir (p< 0.001). Dikdörtgen kesitli NiTi teller en yüksek* OD_{570} *ve CFU değerlerini gösterirken; Cu–NiTi (her iki kesit) ve yuvarlak kesitli SS teller en düşük bakteri yüklerini göstermiştir (p< 0.001). Aynı tel materyalleri içinde, dikdörtgen kesitli tellerde biyofilm birikiminin yuvarlak kesitli tellere kıyasla daha fazla olduğu gözlenmiştir (p< 0.001).*

Sonuç: *Tel materyalinin bileşimi ve geometrisi, ortodontik ark telleri üzerindeki bakteriyel adezyonu birlikte etkilemektedir. Cu–NiTi teller, bakırın muhtemel antimikrobiyal etkisi sayesinde daha düşük biyofilm birikimi göstermiştir. Elde edilen tüm bu bulgular, ortodontik tedavi sırasında mikrobiyal kolonizasyonu en aza indirgemek için materyal seçimi ve tel tasarımının önemini vurgulamaktadır.*

Anahtar Kelimeler: *Biyofilm; Bakteriyel adezyon; Bakır; Ortodontik tel; Streptococcus mutans*

INTRODUCTION

Fixed orthodontic appliances create new retention sites within the mouth that favor the colonization of bacteria and create an increased likelihood of enamel de-mineralization, gingivitis, and white spot lesions throughout treatment[1]. In particular, the placement of brackets and the use of ligatures and archwires inhibit normal oral cleaning processes and thereby promote the adhesion of *Streptococcus mutans* and other cariogenic species, facilitating the formation of mature biofilm[2,3]. Because the early stages of biofilm formation can take hours, the relationship between bacterial species and orthodontic materials has become a major focus of research in preventive orthodontics[4].

Archwires are the most significant orthodontic appliance or element influencing the biofilm, since they are constantly exposed to the oral environment, and fed by interaction with foodstuffs and saliva[5]. The characteristics of the material from which archwires are constructed influence various properties, including surface roughness, surface free energy, and corrosion, which in turn directly affect bacterial adhesion and colonization[6]. Stainless steel (SS) wires are tough and mechanically strong, but also susceptible to corrosion, which may increase surface roughness and encourage bacterial accumulation[7]. On the other hand, nickel–titanium (NiTi) alloys exhibit high elasticity and corrosion resistance, but the high surface free energy of the titanium oxide layer tends to promote microbial adhesion[8].

Proposed methods for combining copper with NiTi alloys (Cu-NiTi) indicate that mechanical rigidity may be improved and that potential antibacterial properties may be conferred via the oligodynamic effects of copper[9,10]. The Cu ions may interfere with bacterial enzymes and affect the integrity of the cell wall, resulting in reduced viability of *S. mutans* on surface of orthodontic appliances[11,12]. Previous reports showing differences in the antibacterial activities of the Cu-NiTi alloy over other alloys, lead to various conclusions that may be accounted for by differences in surface finish, sterilization processes, or experimental design[9,10,13].

Additionally, the surface geometry of archwires also influences microbial adhesion. Specifically, rectangular archwires have a larger surface area and sharper corners than smooth round archwires, providing microenvironments conducive to bacterial colonization[14]. In addition, the salivary deposits and nutrients within these niches make for an especially nutrient-laden atmosphere for greater development and maturation of plaque, and acid production[15]. Conversely, smooth or low roughness surfaces are related to reduced microbial load and ease of plaque release[16,17].

When reviewing studies conducted in previous years, it was observed that the effect of archwire material type and cross-sectional geometry on bacterial adhesion has not been sufficiently clarified[7,10,18-23]. Since orthodontists select archwires based on alloy composition and cross-sectional shape for different types of treatment, understanding how these two factors interact is crucial for interpreting bacterial adhesion and biofilm behavior. In this context, our study will provide guidance on archwire selection to minimize plaque accumulation during orthodontic treatment.

The null hypotheses of this study are stated as follows: H_{01}: There is no significant difference in *S. mutans* biofilm formation and bacterial adhesion among the three orthodontic wire materials and H_{02}: There is no significant difference in *S. mutans* biofilm development and bacterial adhesion between the two cross-sectional wire configurations.

MATERIALS and METHODS

Study Design and Sample Preparation

The present study utilized in vitro methods to evaluate the adhesion of *S. mutans* on orthodontic archwires (SS, NiTi, and Cu-NiTi) that were factory-manufactured and supplied directly in their original packaging, without any mechanical processing or alteration. The six samples under consideration were created in two wire cross-section shapes: round (0.016 inches) and rectangular (0.016 inches x 0.022 inches). Schematic drawings illustrating the cross-sections of the arch wires utilized in the study are presented in Figure 1. These shapes thus created six wire groups. The wire samples are described in Table 1, which includes their specifications, elemental compositions, and manufacturers.

The wire samples (American Orthodontics, USA) were retrieved from their sealed packaging in a laminar airflow cabinet, under sterile conditions. Subsequently, each wire sample was bisected using sterile orthodontic cutters. Subsequently, 1-cm sections were meticulously extracted from both extremities of the specimen to circumvent potential surface contamination arising from the stresses imposed during the manufacturing process of the wire. Consequently, the final test length of each sample was 1 cm.

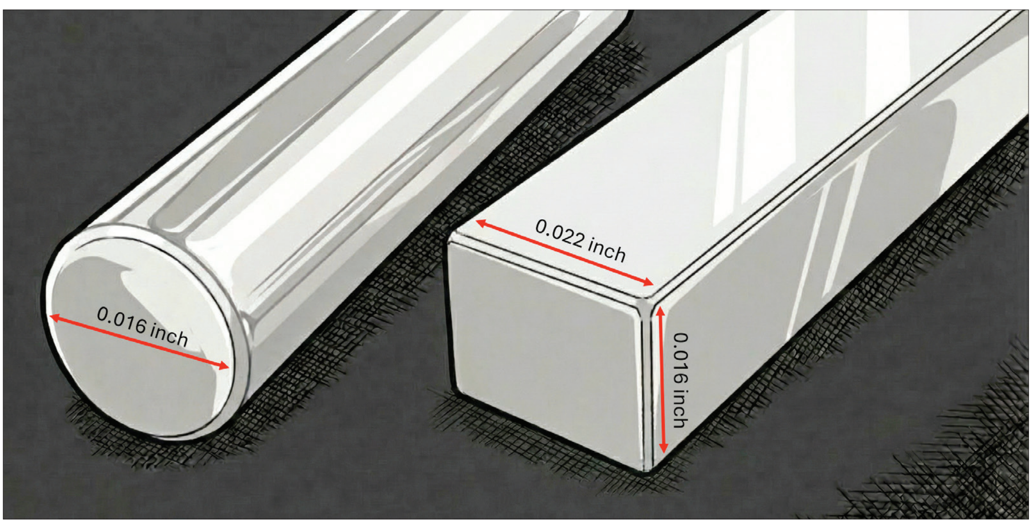

Figure 1. Schematic drawings illustrating the cross-sections of the arch wires utilized in the study.

Table 1. Specifications of orthodontic archwires (both round and rectangular) used in this study

Wire Type	Brand / Manufacturer	Material Composition (% by weight)	Main Elements	Manufacturing Details
Stainless Steel (SS)	American Orthodontics – Master Series / T3 (LOT numbers: L31592 and 348970)	Fe (bal.), Cr (11.5–22), Ni (0–15), Mo (0–6.5), Mn (0–2), Si (0–2), Cu (0–5), Ti (0–0.7), C (0–1.2), Nb (0–0.6)	Iron, Chromium, Nickel	Austenitic stainless steel; non-magnetic; used for fixed and functional orthodontic appliances.
Nickel–Titanium (NiTi)	American Orthodontics – Therma-Ti / Tritanium (LOT numbers: 350206 and P0541043)	Ni (45–60), Ti (40–50)	Nickel, Titanium	Superelastic and shape-memory alloy; provides continuous low force and high elasticity.
Copper Nickel–Titanium (Cu-NiTi)	American Orthodontics – Copper Ni-Ti (LOT numbers: P0511671 and P0502654)	Ni (~47–50), Ti (~44–46), Cu (4–6), Cr (<1)	Nickel, Titanium, Copper	Thermo-active wire providing lower loading forces and temperature-dependent superelasticity.

Note: "bal." denotes "balance," indicating that the remaining portion of the alloy composition consists predominantly of that element.

Power Analysis

A power analysis was performed using G*Power (v3.1.9.7) based on the mean and standard deviation values reported by Oliveira et al. for biofilm accumulation on different orthodontic wire types[18]. Assuming six groups, an alpha of 0.05, and a power of 0.80, the calculation indicated that a minimum of 10 samples per group would be required and therefore 12 samples were included in each group to increase reliability, resulting in a total of 72 samples.

Sterilization Procedure

Each wire sample was first submerged in 70% ethanol for 10 minutes. Thereafter, the samples were rinsed with sterile DI water on two occasions and then dried in a biosafety cabinet. Because the fact that autoclaving has been shown to modify the thermal mechanical properties of wire samples, the samples were exposed to UV light for 30 minutes on each side, as opposed to being autoclaved[24].

Microorganism and Culture Conditions

S. mutans (ATCC 25175) was selected as the test microorganism because of its primary role in the etiology of enamel demineralization during orthodontic treatment. Stock cultures were maintained at –80 °C in BHI broth containing 20% glycerol. Before experimentation, bacteria were reactivated on BHI agar plates and incubated at 37 °C with 5% CO_2 for 48 h.

An overnight culture was prepared by transferring a single colony into 5 mL BHI broth and incubating for 16 h under the same conditions. The culture density was adjusted spectrophotometrically to an optical density of OD_{600}= 1.0, corresponding to approximately 1.5×10^8 colony-forming units/mL (CFU/mL).

Biofilm Formation on Orthodontic Wires

Biofilm formation was induced using a sucrose-enriched static incubation model widely applied in orthodontic biofilm studies[8,18]. Each sterilized wire specimen was put into an individual sterile glass tube containing 500 µL of standardized *S. mutans* suspension (OD_{600} = 1.0; approximately 1.5×10^8 CFU/mL) and 5 mL of BHI broth supplemented with 3% sucrose. The sucrose-enriched medium was used to enhance the synthesis of extracellular polysaccharides by *S. mutans*, thereby supporting early biofilm formation under controlled in vitro conditions.

Each tube was incubated at 37 °C in a 5% CO_2 for 24 hours. After incubation, each wire was gently rinsed once with phosphate-buffered saline (PBS, pH 7.4) to remove loosely attached planktonic cells.

Biofilm Biomass Quantification by Crystal Violet Staining

Biofilm biomass was quantified using the crystal violet method, following established protocols[8,18]. After PBS rinsing, wires were transferred to sterile petri dishes and washed three times with PBS. The wire samples were then fixed in 96% ethanol for 15 minutes, followed by three washes with distilled water. The bound dye was subsequently dissolved by placing each wire sample in 1 ml of 33% acetic acid and gently mixing the wire samples for 10 minutes. Subsequently, 100 µL aliquots of the solubilized dye from each sample were transferred, in duplicate, into a 96-well microtiter plate. Absorbance at 570 nm was measured using a Dynex DS2 microplate reader (Dynex Technologies, USA). Mean OD_{570} nm values were used for quantitative comparison between the experimental groups.

Viable Bacterial Counts

Viable adherent bacteria were quantified using a vortex–sonication detachment and plating method consistent with prior studies assessing *S. mutans* adhesion on orthodontic materials[22]. To assess bacterial viability, each wire was transferred into a sterile tube containing 1 ml of PBS. Attached bacteria were released by vortexing for 30 seconds, followed by five minutes of ultrasonication. The suspensions were serially diluted ten-fold in sterile PBS, and 100 µL of appropriate dilutions was plated, in duplicate, onto BHI agar. Plates were incubated at 37 °C with 5% CO_2 for 48 hours, and CFU were counted.

The laboratory stages of the in vitro study are illustrated in the flow chart presented in Figure 2.

Statistical Analysis

Data were analyzed using IBM Statistical Package for the Social Sciences v23 (IBM Corp., Armonk, NY, USA) and Jamovi v2.7.6 (The Jamovi Project, Sydney, Australia). The normality of the data distribution was evaluated using the Shapiro–Wilk test. For variables that did not show a normal distribution, comparisons between wire material and cross-sectional form were performed using Robust analysis of variance (ANOVA). When significant differences were detected, Holm-adjusted Robust t-tests were applied for multiple pairwise comparisons. The relationships between non-normally distributed quantitative variables were examined using Spearman's rho correlation. All quantitative data were presented as median (minimum–maximum) in addition to

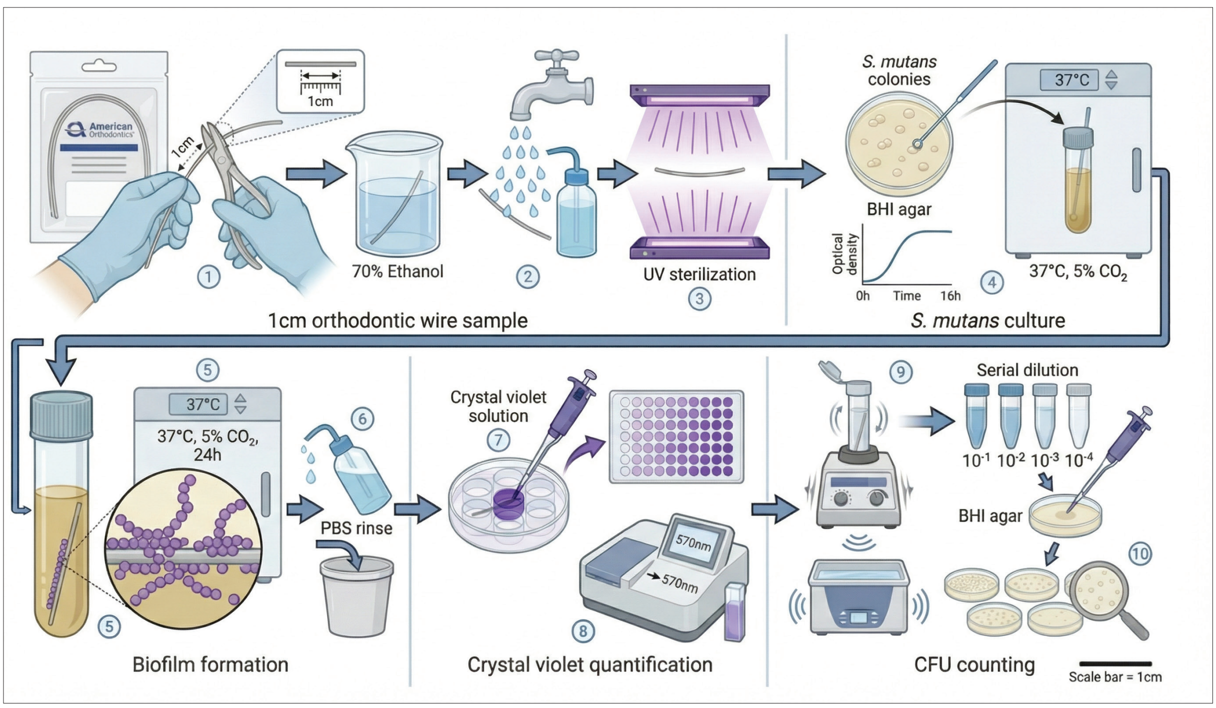

Figure 2. Flowchart illustrating the laboratory stages of the in vitro study.

mean ± standard deviation (SD) values. A p-value of <0.05 was considered statistically significant.

RESULTS

Biofilm Optical Density (OD_{570})

Robust ANOVA revealed a statistically significant difference in biofilm formation among the six wire groups ($p < 0.001$, $\eta^2 = 0.834$). Both wire material and cross-sectional geometry had a significant effect on *S. mutans* biofilm accumulation. Mean ± SD and median biofilm optical density values for each group are summarized in Table 2 and illustrated in Figure 3.

Rectangular NiTi wires exhibited the highest OD_{570} values, with a mean of 0.057 ± 0.005 and a median value of 0.059 (0.050–0.064). In contrast, the lowest biofilm accumulation was observed in the round SS [0.006 ± 0.001, median 0.006 (0.005–0.009)] and round Cu–NiTi [0.006 ± 0.002, median 0.005 (0.004–0.008)] groups. Intermediate levels of biofilm formation were detected in NiTi round, SS rectangular, and Cu–NiTi rectangular groups.

Holm-adjusted pairwise comparisons confirmed that rectangular NiTi wires differed significantly from all other groups ($p < 0.05$), while no statistically significant difference was found between round SS and round Cu–NiTi wires ($p > 0.05$).

Within the same alloy type, rectangular wires exhibited higher biofilm accumulation than round wires, indicating that cross-sectional geometry markedly influences bacterial adherence.

These findings indicate that rectangular NiTi wires are substantially more susceptible to *S. mutans* biofilm formation than stainless steel and Cu–NiTi wires, highlighting the combined influence of both alloy composition and surface geometry on microbial adhesion.

Viable Bacterial Counts (CFU/mL)

A significant effect of wire material, cross-sectional form, and their interaction on viable bacterial adhesion was also observed ($p < 0.001$, $\eta^2 = 0.845$). Mean ± SD and median CFU values for each group are summarized in Table 3 and illustrated in Figure 4.

Table 2. Comparison of Biofilm optical density (OD570) values among different wire materials and cross-sectional forms

Cross Section	Groups: NiTi	SS	Cu-NiTi	Overall		Test Statistic	df_1	df_2	p^x	η^2
Round	0,015 (0.006 - 0.017)^A 0.014 ± 0.004	0.006 (0.005 - 0.009)^C 0.006 ± 0.001	0.005 (0.004 - 0.008)^C 0.006 ± 0.002	0.007 (0.004 - 0.017) 0.009 ± 0.004	Material	148.000	2	66	<0.001	0.818
Rectangular	0.059 (0.05 - 0.064)^B 0.057 ± 0.005	0.013 (0.006 - 0.018)^A 0.012 ± 0.004	0.014 (0.008 - 0.02)^A 0.013 ± 0.004	0.015 (0.006 - 0.064) 0.027 ± 0.021	Cross Section	206.000	1	66	<0.001	0.692
Overall	0.034 (0.006 - 0.064)^a 0.035 ± 0.022	0.008 (0.005 - 0.018)^b 0.009 ± 0.004	0.008 (0.004 - 0.02)^b 0.01 ± 0.005	0.011 (0.004 - 0.064) 0.018 ± 0.018	Material x Cross Section	166.000	2	66	<0.001	0.834

^{x}Robust ANOVA. Values are expressed as median (min–max) / mean ± SD.
Groups sharing the same uppercase letters ($^{A–B}$) are not significantly different among wire types; groups sharing the same lowercase letters ($^{a–c}$) indicate no significant interaction differences. η^2: Partial Eta Squared, df_1: Degrees of freedom for the effect, df_2: Degrees of freedom for the error.

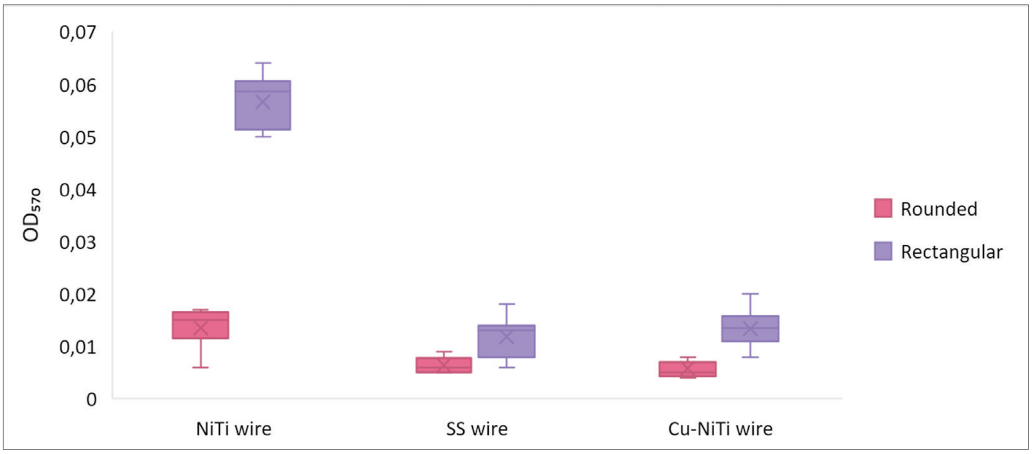

Figure 3. Boxplot representation of *S. mutans* biofilm formation (OD570) on different orthodontic wire materials and cross-sectional forms.

NiTi rectangular wires showed the highest bacterial load, with a mean of 2.049 x 10^5 ± 0.128 x 10^5 CFU/mL and a median of 2.03 x 10^5 (1.92 x 10^5–2.40 x 10^5), followed by NiTi round wires [1.975 x 10^5 ± 0.136 x 10^5, median 2.00 x 10^5 (1.80 x 10^5–2.30 x 10^5)].

In contrast, the lowest bacterial counts were observed in the round SS [0.221 x 10^5 ± 0.101 x 10^5, median 0.21 x 10^5 (0.11 x10^5–0.40 x 10^5)] and round Cu–NiTi [0.202 x 10^5 ± 0.098 x 10^5, median 0.15 x 10^5 (0.11 x 10^5–0.40 x 10^5)] groups, both showing markedly reduced viable bacterial adhesion compared to NiTi wires and all rectangular wires.

Holm-adjusted post-hoc analysis indicated that both NiTi groups (round and rectangular) differed significantly from all other groups ($p< 0.05$), whereas no significant difference was found among SS round, Cu–NiTi round, and Cu–NiTi rectangular wires ($p> 0.05$).

Within the same alloy type, rectangular wires generally showed slightly higher bacterial retention than round wires, although this difference was not statistically significant for NiTi.

These results confirm that Cu–NiTi and stainless-steel wires demonstrate the most favorable antibacterial surface properties, whereas NiTi wires—particularly with rectangular cross-section—show the greatest susceptibility to *S. mutans* adhesion and viability.

DISCUSSION

Fixed orthodontic appliances create a retentive oral environment that promotes the adhesion of early Streptococci colonizers—particularly *S. mutans*. Through glucosyl-transferase activity, these bacteria form extracellular polysaccharides and mature biofilms, increasing the risk of demineralization and white spot lesions[19,25]. Biofilm formation is strongly affected by surface roughness and free energy, and even slight increases in roughness can create protective niches that enhance bacterial virulence[26,27]. It is therefore hypothesized that the alloy composition and geometric characteristics of orthodontic archwires are potential factors influencing the total microbial load during treatment[8,20].

Copper is well known for its broad-spectrum antimicrobial activity, disrupting bacterial membranes and metabolic pathways[28]. This has led to the hypothesis that copper-containing orthodontic alloys may influence early biofilm formation[29]. Also, microstructural and metallurgical differences between NiTi and Cu–NiTi influence friction and roughness, thereby indirectly affecting microbial adhesion[30]. Beyond physical and chemical factors, ion release and biocorrosion occur bidirectionally between archwires and oral microflora. *S. mutans* can modify local redox and pH conditions, influencing metal dissolution and surface integrity, thereby creating feedback loops that affect adhesion and viability[7,21,31]. When considered as a whole,

Table 3. Comparison of viable bacterial counts (CFU/mL ×10^5) among different wire materials and cross-sectional forms

Cross Section	Groups									
	NiTi	SS	Cu-NiTi	Overall		Test Statistic	df1	df2	p^x	η^2
Round	2 (1.8 - 2.3)^A 1.975 ± 0.136	0.21 (0.11 - 0.4)^B 0.221 ± 0.101	0.15 (0.11- 0.4)^B 0.202 ± 0.098	0.3 (0.11- 2.3) 0.799 ± 0.85	Material	694.000	2	66	**<0.001**	0.955
Rectangular	2.03 (1.92 - 2.4)^A 2.049 ± 0.128	1 (1 - 1.2)^C 1.041 ± 0.063	0.975 (0.12 - 1.12)^C 0.896 ± 0.262	1.045 (0.12 - 2.4) 1.329 ± 0.546	Cross Section	240.000	1	66	**<0.001**	0.784
Overall	2 (1.8 - 2.4)^a 2.012 ± 0.134	0.7 (0.11- 1.2)^b 0.631 ± 0.427	0.37 (0.11 - 1.12)^b 0.549 ± 0.404	1 (0.11 - 2.4) 1.064 ± 0.758	Material x Cross Section	180.000	2	66	**<0.001**	0.845

^{x}Robust ANOVA. All values are expressed as ×10^5 CFU/mL. Values are expressed as median (min–max) / mean ± SD.
Groups sharing the same uppercase letters ($^{A-B}$) are not significantly different among wire types; groups sharing the same lowercase letters ($^{a-c}$) indicate no significant interaction differences. (η^2): Partial Eta Squared, df_1: degrees of freedom for the effect, df_2: degrees of freedom for the error.

this framework indicates that the selection of alloy (i.e. SS, NiTi, Cu–NiTi) and the geometry of the cross-section can serve as mechanistic variables that are associated with patient-specific biofilm risk and preventative protocols[9,22,23].

The findings of this in vitro study demonstrated that both wire material type and cross-sectional geometry exerted a significant effect on *S. mutans* biofilm formation and bacterial viability, thereby leading to the rejection of both null hypotheses. Among the groups, rectangular NiTi wires exhibited the highest biofilm accumulation, whereas round SS and Cu–NiTi wires showed the lowest. Similarly, the highest viable bacterial counts were recorded for both round and rectangular NiTi wires, while round SS and Cu–NiTi wires demonstrated minimal bacterial viability.

The markedly increased bacterial adherence observed for NiTi wires relative to SS or Cu-NiTi is consistent with earlier work showing that *S. mutans* adheres more strongly to NiTi because of surface energy considerations and increased reactivity in aqueous environments[22]. Conversely, SS wires maintain a chemically stable chromium oxide film, thereby minimizing corrosion and surface changes, and consequently reducing bacterial colonization[32]. These disparities in surface chemistry partially elucidate the considerably diminished CFU numbers observed for SS in the present study.

In a four-week clinical in vivo study undertaken by Abraham et al., greater *S. mutans* adhesion occurred on Cu–NiTi than on NiTi, with a concomitant increase in the surface roughness and surface free energy (SFE) of Cu–NiTi after intraoral use[9]. These results were contrary to those previously found in vitro by the present authors, in which *S. mutans* adhesion to Cu–NiTi was shown to be low. It is reasonable to hypothesize that the apparent discrepancy can be attributed to two possible explanations. First, intraoral ageing (salivary pellicle formation, masticatory forces, dietary exposure, and pH cycling) may be responsible for increases in roughness and SFE in alloys with differing chemical compositions. This increase in roughness and SFE may consequently confer an advantage for bacterial adhesion, irrespective

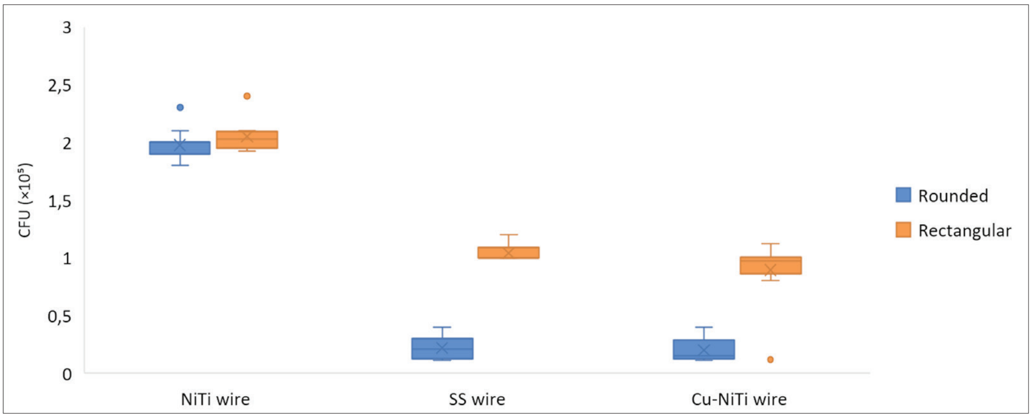

Figure 4. Boxplot showing viable bacterial counts (CFU × 10^5) on different orthodontic wire materials and cross-sectional forms.

of chemical equilibrium. Second, quantitative polymerase chain reaction (qPCR) analysis of the wires allows highly sensitive quantification of adherent bacterial DNA, including non-culturable or partially damaged cells that CFU methods cannot detect. The referenced study showed that all wire types exhibited increased roughness and surface free energy after four weeks, supporting the notion that time-dependent changes enhance the surface's propensity for microbial adhesion in the oral environment.

Hepyukselen et al., in a prospective study evaluating SE NiTi, CuNiTi, and TMA wire types, compared microbial colonization using clinical periodontal indices, culture methods, and PCR analysis[33]. They found that microbial retention was lower in CuNiTi wires after the rinsing period, which may be related to the potential antimicrobial effect of copper.

Titiz et al. conducted an in vitro experiment to measure the adhesion of *S. mutans* on stainless-steel, nickel-titanium and copper-nickel-titanium arch wires[34]. The findings of the experiments demonstrated that there was no significant difference between the three materials in terms of bacterial adhesion. The authors also noted that bacterial presence caused corrosive changes on the wire surface.

In partial concurrence with the findings presented herein, Oliveira et al. conducted a comparative analysis of biofilm formation among various aesthetic and metallic orthodontic archwires, using *S. mutans* cultures exposed to varying concentrations of sucrose[18]. The researchers discovered that crystalline violet staining increased significantly only on NiTi micro-dental white archwires in 3% sucrose, whereas biofilm formation on Cu-NiTi wires was reduced under similar conditions. This finding corroborates those of the present study, which indicate that the copper component may exert a slight inhibitory effect on *S. mutans* adhesion and biofilm formation, at least in the early stages of colonization. It was hypothesized that the antibacterial properties of the copper ion, in conjunction with its capacity to modify the integrity of the cell membrane and the function of protein, could provide a rationale for this diminished retention of bacteria. However, the extent of this effect may vary depending on certain conditions, such as the presence of sucrose and the duration of exposure.

In relation to the geometry of orthodontic arch wires, Abraham et al. also reported that rectangular arch wires exhibited significantly more *S. mutans* adhesion compared to round wires[9]. Furthermore, it was observed that rectangular cross-sections exhibited a greater plaque retention area at the bracket-wire interface in comparison to round cross-sections. This finding is consistent with the results of our study, which demonstrated that rectangular shapes exhibited significantly more bacterial retention compared to round cross-sections, independent of the material. The enhanced adhesion exhibited by rectangular wires can be ascribed to their augmented surface area and more pronounced edges.

Limitations

The present in vitro study was conducted within the confines of standardized laboratory conditions. It is acknowledged that such conditions may not necessarily emulate the complex and dynamic environment of teeth and the oral cavity in vivo. It is hypothesized that salivary pellicle formation, enzymatic activity, variation in denudation patterns and incidence of mechanical stress would have the effect of increasing or decreasing bacterial adhesion and biofilm formation. Furthermore, this study was limited to a single bacterial species, namely *S. mutans*. While this bacterium is considered the primary cariogenic microorganism, a wide variety of bacteria proliferate in the oral environment during orthodontic treatment. Another limitation was the relatively short incubation period of 24 hours, which corresponds to the early stage of biofilm formation. It is recommended that subsequent studies include longer incubation periods and conduct in vivo research to more accurately mimic the clinical scenario.

CONCLUSION

The findings clearly indicate that both archwire material composition and cross-sectional geometry exert a significant influence on *S. mutans* biofilm formation and bacterial viability. Among the materials tested, copper–nickel–titanium (Cu–NiTi) wires demonstrated a relatively lower degree of bacterial adhesion compared with conventional NiTi and stainless-steel wires, suggesting that the antimicrobial activity of copper ions may provide a modest yet measurable protective effect during the early stages of biofilm formation. Conversely, rectangular cross-section wires consistently exhibited higher optical density (OD_{570}) and viable bacterial counts than their round counterparts, underscoring the importance of wire geometry in promoting microretentive areas conducive to bacterial colonization. Clinically, careful selection of wire type—particularly in patients with poor oral hygiene or high caries risk—may contribute to reducing enamel demineralization and improving long-term periodontal health.

ETHICS COMMITTEE APPROVAL

This study was approved by the İnönü University Health Sciences Scientific Researchs Ethics Committee (Decision no: 2025/8183, Date: 29.07.2025).

CONFLICT of INTEREST

The authors have no conflicts of interest to declare that are relevant to the content of this article.

AUTHORSHIP CONTRIBUTIONS

Concept and Design: SÖ

Analysis/Interpretation: SÖ, EST

Data Collection or Processing: EST, MKK

Writing: SÖ, EST

Review and Correction: All of authors

Final Approval: All of authors

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