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Original Article

Nickel Release and the Viability of *Streptococcus mutans* Corresponding to Low Risk of Dental Caries in Artificial Saliva Containing Orthodontic Appliances: In Vitro Study

Serap Titiz[1], Zalike Keskin Erdoğan[2], Elif Esin Hames Tuna[3], Aynur Aras[4]

[1]Department of Orthodontics, Uşak University, Faculty of Dentistry, Uşak, Turkey
[2]Division of Biomaterials and Tissue Engineering, Eastman Dental Institute, University College London, London, United Kingdom
[3]Department of Bioengineering, Ege University, Faculty of Engineering, İzmir, Turkey
[4]Department of Orthodontics, Ege University, Faculty of Dentistry, İzmir, Turkey

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Main Points

- Corrosion is an inevitable natural process in fixed orthodontic appliances.
- The observed decrease in the growth of *Streptococcus mutans* was likely caused by corrosion or a related process.
- Even a low level of *S. mutans* represents a corrosion-promoting factor for stainless steel-based materials.
- The corrosion behavior and biocompatibility of the studied alloys might depend on their surface roughness.

ABSTRACT

Objective: The aims of this study were to determine the effect of different levels of *Streptococcus mutans* that correspond to a low risk of dental caries on nickel release and to determine the viability of *S. mutans*.

Methods: Simulated fixed orthodontic appliances composed of copper nickel titanium, nickel titanium, or stainless steel were immersed in Klimek artificial saliva for 10 days with or without *S. mutans* inoculation on day 7. Same levels of *S. mutans* cultures (4×10^4 cfu/mL) were inoculated into the artificial saliva without orthodontic appliances. Nickel release was detected by inductively coupled plasma mass spectrometry. The archwire surface was analyzed by atomic force microscopy and scanning electron microscopy.

Results: The density of *S. mutans* significantly increased in the artificial saliva without orthodontic appliances ($P < .05$). Appliances with nickel titanium alloys showed higher nickel release in the artificial saliva with or without *S. mutans* than those with copper nickel titanium or stainless steel archwires ($P < .05$). However, *S. mutans* increased nickel release only in orthodontic appliances with stainless steel archwires ($P < .05$). Although atomic force microscopy showed that the surface of as-received stainless steel archwires was smoother than that of nickel titanium or nickel titanium archwires, *S. mutans* increased the surface roughness of only the SS archwires. *S. mutans* adhered to all archwire types.

Conclusion: While corrosion or corrosion-related processes may have decreased the growth capacity of *S. mutans*, reciprocally, *S. mutans* influenced corrosion. Rough surfaces can also promote corrosion; therefore, the surface roughness of metal alloy orthodontic appliances should be evaluated to determine their corrosion behavior.

Keywords: Corrosion, dental caries, nickel release, orthodontic appliance, risk, Streptococcus mutans

INTRODUCTION

Metals or metal alloys are corroded due to oxidation or other chemical effects, and ions are released into the environment as a result. Metals become corroded in the oral environment within 7 days, after which corrosion decreases, and then stops.[1,2] This process is induced by the development of new corrosion factors. The cycle will

Corresponding author: Serap Titiz, e-mail: dtseraptitiz79@hotmail.com

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continue depending on the microbiological, enzymatic, ionic, and thermal properties in the oral environment and can cause the metal to corrode and degrade biologically. Therefore, corrosion is an inevitable natural process in fixed orthodontic appliances. Most orthodontic treatments are implemented using fixed devices such as brackets, tubes or bands, and wires made of metal alloys. Nickel (Ni) and chromium (Cr) are the primary ions released from these alloys. Ni and Cr are post-corrosion products that can have genotoxic, mutagenic, and cytotoxic effects that could cause contact allergies, asthma, hypersensitivity, birth defects, and reproductive damage.[3,4] Thus, the biocompatibility of the materials utilized in orthodontic treatments is of importance.

Streptococcus mutans is a gram-positive, facultative anaerobic bacterium found mostly in the human oral cavity and is involved in the formation of dental caries. Hence, *S. mutans* is graded according to the colony-forming units per milliliter (cfu/mL) in the mouth. The risk of caries is graded as high ($\geq 10^6$ cfu/mL), moderate (10^5-$<10^6$ cfu/mL), and low ($\leq 10^5$).[5] Orthodontic treatments should be implemented after the completion of all essential dental and periodontal therapies. Difficulties with brushing the teeth and an increase in areas of retention during fixed orthodontic treatment might increase the density of *S. mutans*, thereby increasing the risk of caries. However, it is unlikely for patients with good oral hygiene to have a high density of *S. mutans* during orthodontic treatment. Studies have reported that the corrosion of alloys such as cobalt (Co), Cr and nickel-chromium (Ni/Cr), and titanium (Ti) dental implants increases in the presence of *S. mutans*, which increases the risk of caries.[6-10] However, to the best of our knowledge, the in vitro corrosion behavior of alloys under a low risk of caries has not yet been investigated. The corrosion process during orthodontic treatment in a mouth with a relatively low risk of dental caries could inhibit the growth of *S. mutans*. Thus, in the present study, the primary objective was to determine the amount of Ni released by simulated orthodontic appliances with different types of archwires in vitro in the presence of *S. mutans* at levels that correspond to a low risk of caries and the secondary objective was to determine the growth ability of *S. mutans* in a corrosive environment.

METHODS

This was an in vitro study. Fixed orthodontic appliances representing half of the maxillary arch consisted of 5 structurally identical brackets, a molar band, and 6-cm-long copper-nickel-titanium (CuNiTi), nickel-titanium (NiTi), or stainless steel (SS) archwires tied with elastic ligatures (Astar Orthodontics Inc., Shanghai, China). Klimek artificial saliva comprising ascorbic acid (0.002 g), glucose (0.030 g), NaCl (0.580 g), $CaCl_2$ (0.170 g), NH_4Cl (0.160 g), KCl (1.270 g), NaSCN (0.160 g), KH_2PO_4 (0.330 g), urea (0.200 g), Na_2HPO_4 (0.340 g), and mucin (2.700 g) (Bacto-Mucin Bacteriological) in 1 L of distilled water (pH 6.75) was prepared.[11] In this study, there was 1 experimental group (EG) and 2 control groups (CG 1 and CG 2). The EG and CG 1 groups were subgrouped according to appliances with CuNiTi (group A), NiTi (group B), and SS (group C) archwires. All EG and CG 1 subgroups consisted of 3 replicates. All simulated fixed orthodontic appliances were immersed in 50 mL of Klimek artificial saliva. The artificial saliva in the EG (3 appliances each in group A [C], group B [C], and group C [C]) groups was inoculated with 100 µL of *S. mutans* (ATCC 25175; 4×10^4 cfu/mL) on day 7 of the experiment. The other half of the simulated fixed orthodontic appliances (3 appliances each in group A [C], group B [C], and group C [C]) remained submerged in the Klimek artificial saliva until day 10 without *S. mutans* inoculation in CG 1. The CG 2 group comprised 9 replicates; Klimek artificial saliva (without appliances) was inoculated with the same level of *S. mutans* under identical conditions. The samples were incubated at 37°C in a 5% CO_2 atmosphere for 72 h, following which *S. mutans* was evaluated (cfu/mL) using the spread plate technique. The initial and final pH values of Klimek artificial saliva were measured at room temperature using an HI-1131B pH meter (Hanna Instruments Inc., Carrollton, Tex, USA). The experimental setups and sample preparation for inductively coupled plasma mass spectrometry (ICP-MS) were made by S. Titiz, Z.K. Erdogan. Figure 1 shows a brief schema of the experiment.

Detection of Nickel Release by Inductively Coupled Plasma Mass Spectrometry (ICP-MS)

The amount of Ni released into Klimek artificial saliva was evaluated using an ELAN DRC-eICP-mass spectrometer (Perkin Elmer,

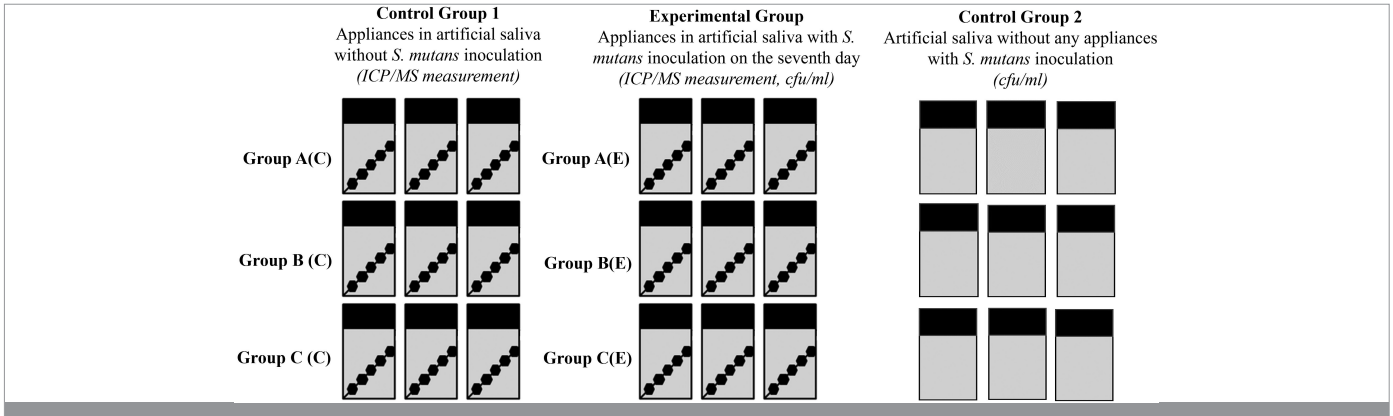

Figure 1. Schema of experimental setup. Subgroups of the experimental group: appliances with archwires containing CuNiTi (group A [E]), NiTi (group B ([E]), and SS (group C [E]). Subgroups of control group 1: appliances with archwires containing CuNiTi (group A [C]), NiTi (group B [C]), and SS (group C [C]). Control group 2: artificial saliva with *Streptococcus mutans* inoculation

Norwalk, CT, USA). Samples were placed in a 40-kHz ultrasonic water bath (ISOLAB Laborgeräte GmbH., Wertheim, Germany) for 30 min, centrifuged at 7000 rpm (Optima™ X Series centrifuge; Beckman Coulter, Brea, Calif, USA), and then passed through a 0.45-mm Millex® Syringe filter (Sigma-Aldrich Corp., St. Louis, Mo, USA). Samples of homogenized artificial saliva (500-600 µL) were weighed, immersed in 10 mL of 65% nitric acid (Sigma-Aldrich Corp.) to breakdown organic compounds, and placed in a microwave oven (1600 W, 100% power at 30° and 160 mmHg). The samples were then placed in an oven for measurement, with 50 mL of ultrapure water.

Characterization of Surface Topography

The average roughness (Ra) of the as-received and post-immersion archwires and their surface morphologies were assessed in the EG and CG 1 groups by atomic force microscopy (AFM) and scanning electron microscopy (SEM) (Gemini SEM 300; Carl Zeiss AG., Oberkochen, Germany). Microorganisms were fixed by dehydrating the wires in a graded series of alcohol (50%, 70%, 85%, 90%, and 100%) and immersed in 10% (v/v) glutaraldehyde.

Statistical Analysis

Data are shown as number, mean, and standard deviation. Normal distribution was assessed using Shapiro–Wilk test, and variance homogeneity was evaluated using Levene test. The average Ni oscillation between independent groups satisfying the assumptions was compared using an independent samples *t*-test. The averages of 3 or more independent groups were compared using the one-way ANOVA if they met the assumed criteria and those of 3 independent groups that did not meet the assumption criteria were compared using Kruskal–Wallis test. Groups with differences were assessed with Bonferroni correction. Differences with a ***P***-value < .05 were considered significant.

RESULTS

Findings of ICP-MS

Table 1 and Figure 2a and b show a comparison of Ni-release in EG and CG1. More Ni was released by appliances with NiTi (group B) than with CuNiTi or SS archwires (group A or group C) in CG 1 and EG ($P < .05$). The amount of Ni released from appliances with SS and CuNiTi archwires was similar in CG 1 ($P > .05$), whereas more Ni was released from appliances with SS archwires in EG than from appliances with CuNiTi archwires ($P < .05$). Nickel release between the subgroups of CG 1 and EG was evaluated; it was found that the amount of Ni released between Groups C_C and C_E significantly differed ($P < .05$). Furthermore, *S. mutans* increased the rate of Ni-release from appliances with SS archwires (group C, $P < .05$) but did not significantly affect that from appliances with CuNiTi or NiTi archwires (group A or B; $P > .05$; Table 1, Figure 2b).

Characterization of Surface Topography

Table 2 and Figure 3 show the comparisons of the Ra values of archwires as-received and after immersion in the EG and CG 1 groups. The surface roughness of CuNiTi and NiTi archwires as-received and after immersion in artificial saliva medium with or without *S. mutans* was similar ($P > .05$), and their Ra values were

Table 1. Comparison of the amounts of nickel released between the experimental groups and control group 1

	Group A Mean ± SD	Group B Mean ± SD	Group C Mean ± SD	*P*	A/B *P*	B/C *P*	A/C *P*
CG1	42.52 ± 4.33	68.341 ± 2.657	37.97 ± 2.27	.0001*	.0001*	.000*	.65
EG	37.40 ± 4.28	72.307 ± 5.31	52.45 ±4.23	.000*	<.0001	.006*	.022*
P	.219	.312	.006*				

*Significant difference at $P < .05$. CG1, control group 1; EG, experimental group; SD, standard deviation. Appliances with CuNiTi archwires, group A (A); appliances with NiTi archwires, group B (B); appliances with SS archwires, group C (C).

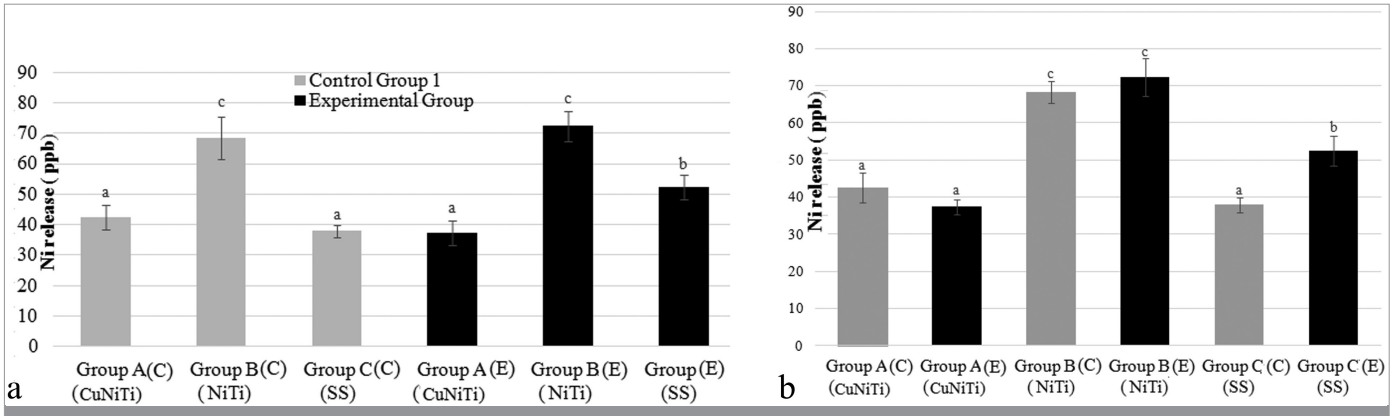

Figure 2. a, b. Nickel release in groups and subgroups. (a) Amount of Ni released in subgroups A_C, B_C, and C_C of control group 1, and A_E, B_E, and C_E of experimental group. Appliances with archwires containing CuNiTi (group A), NiTi (group B), and SS (group C). (b) Amount of Ni released by the control group 1 and experimental group subgroups. a and b indicate statistically significant differences ($P < .05$) between each measurement item compared

Table 2. Comparison of the Ra values between as-received archwires and those in the experimental groups and control group 1

	CuNiTi (A) Mean ± SD	NiTi (B) Mean ± SD	SS (C) Mean ±SD	*P*	A/B *P*	B/C *P*	A/C *P*
As-r	79.28 ± 1.00	78.18 ± 2.00	40.05 ± 1.00	.000*	1.000	.000*	.000*
CG1	80.66 ± 2.51	81.16 ± 1.00	41.66 ± 1.52	.000*	1.000	.000*	.000*
EG	81.00 ± 1.00	78.66 ± 2.77	60.04 ± 1.00	.027*	.534	.025*	.027*
P	.354	.082	.000*				
As-r/CG1			0.352				
As-r/EG			0.000*				
CG1/EG			0.000*				

*Significant difference at $P < .05$. As-r, as-received; CG1, control group 1; EG, experimental group; SD, standard deviation. Appliances with CuNiTi archwires, group A (A); appliances with NiTi archwires, group B (B); appliances with SS archwires, group C (C). Paired comparisons were not performed when *P* (ANOVA) was not significant.

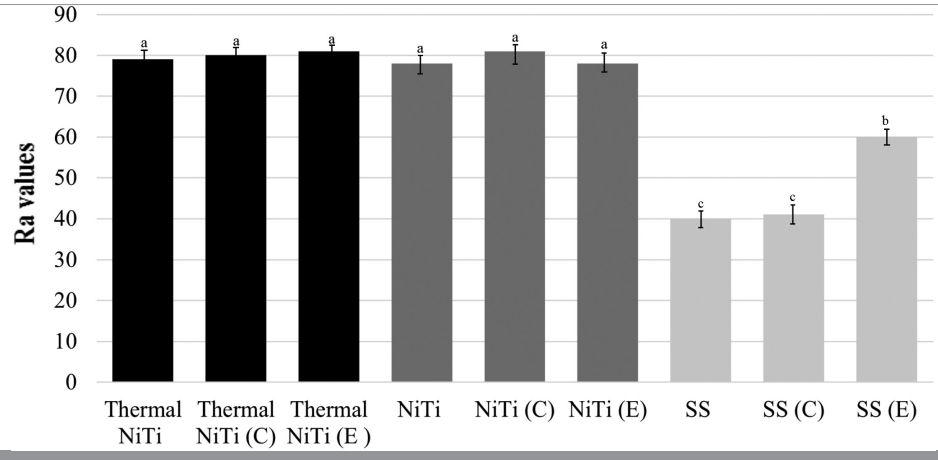

Figure 3. Comparison of the Ra values of archwires as-received and after immersion. Control group 1 (C) and experimental group (E). The first column shows the archwire type. a and b indicate statistically significant differences ($P < .05$) between each compared measurement item

higher than those of SS archwires ($P < .05$). The surface roughness of the CuNiTi and NiTi archwires did not change in the artificial saliva with and without *S. mutans* in the EG and CG 1 groups ($P > .05$). The surface roughness of the SS archwires increased in artificial saliva with *S. mutans* in CG 1 ($P < .05$), and the Ra values of SS archwires in the EG group were higher than those of as-recieved and SS archwires in the CG 1 group ($P < .05$). The AFM findings were consistent with the surface structures visualized by SEM (Figures 4–6).

Streptococcus Mutans Cell Viability

S. mutans proliferated more in artificial saliva in the absence of appliances in the CG 2 group compared with that in the EG group ($P < .05$), whereas the EG subgroups did not significantly differ ($P > .05$; Tables 3 and 4 and Figure 7). The SEM images revealed *S. mutans* adhesion to all types of archwires (Figure 6). The presence of *S. mutans* decreased the pH in the CG 2 group (pH 4.95-4.93), but it did not affect the pH of the EG subgroups (pH 6.75-6.73).

DISCUSSION

Non-optimal conditions in the oral cavity can accelerate corrosion; thus, understanding the corrosion behavior of metals is important to evaluate their biocompatibility. Metal (Ni, Fe, Cu, and Ti) ions released into the environment as a result of corrosion might cause local or systemic adverse reactions in some patients.[4] One of the most prevalent local reactions is sensitivity to Ni, which exerts systemic cytotoxic and mutagenic effects[3]; however, it is widely applied in dentistry. Thus, Ni-release from orthodontic appliances with NiTi, CuNiTi, or SS archwires was investigated in the presence of *S. mutans*. The corrosive behavior of metals or alloys is determined by estimating the number of ions passing into their liquid environment (immersion tests) or is determined electrochemically. However, electrochemically evaluating the corrosion behavior of metals can lead to significantly different results from those obtained in the oral environment.[12] Therefore, the corrosive behavior of metals was determined using immersion tests. In this study, simulated orthodontic appliances composed of 5 structurally identical SS brackets and bands, elastic ligatures, and 3 types of orthodontic wires. The difference in corrosive behavior in the EG and CG 1 subgroups can be attributed to the types of orthodontic wires. Ions passing into artificial saliva can be measured in immersion tests using various devices. Hwang et al.[1] and Kuhta et al.[13] determined the number of corrosion products by ICP/MS as in our study. However, Barret et al.[2] and Reddy[14] determined the number of ions by ICP-optical emission spectroscopy (OES).

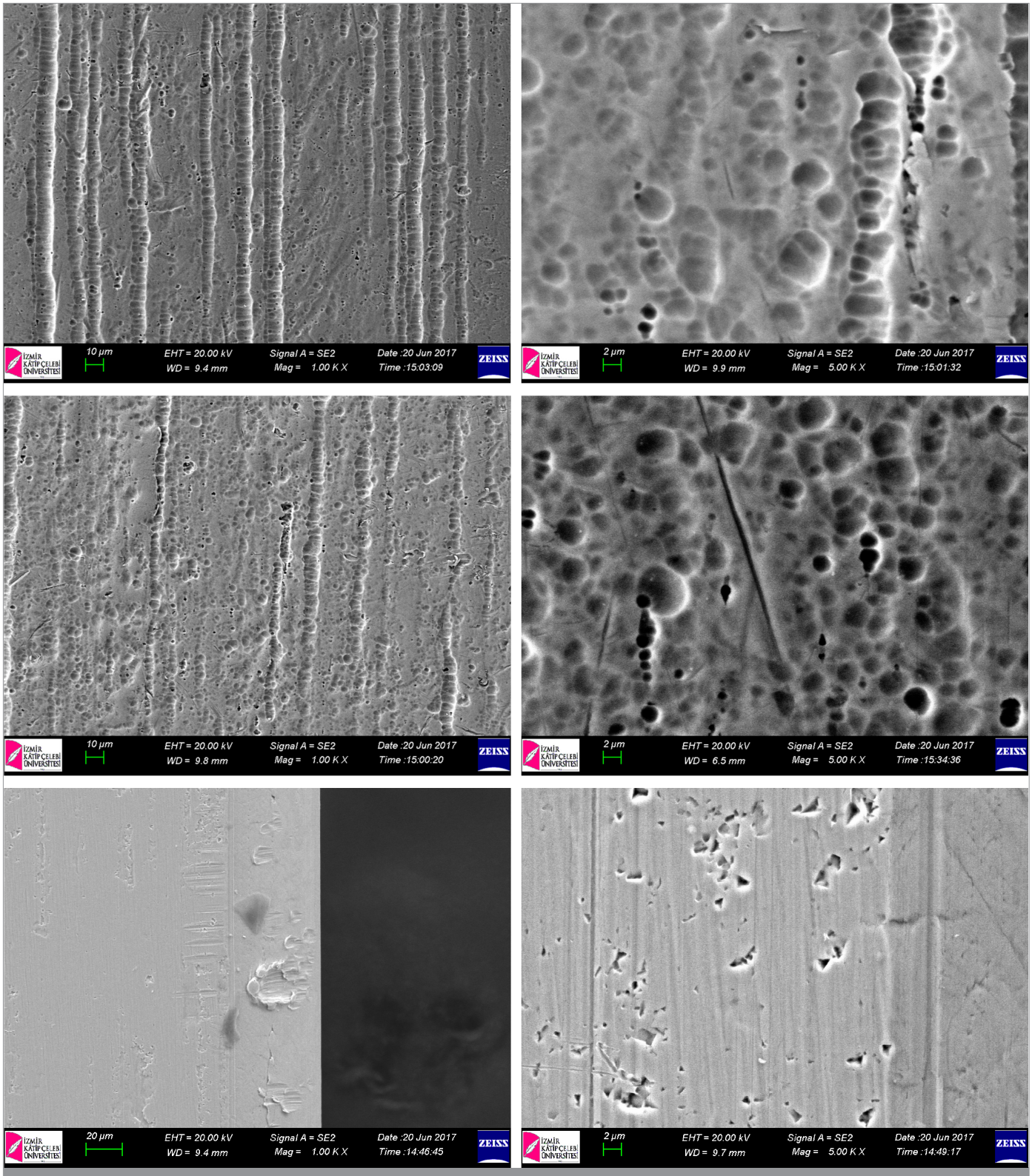

Figure 4. Scanning electron microscopy images of as-received archwires. Subgroups: CuNiTi (a, b), NiTi (c, d), and SS (e, f). Magnification: 1.00 KX (a, c, e) and 5.00 KX (b, d, f)

While ICP-OES is widely available and reasonably cost-effective, ICP-MS requires experienced staff and is expensive; furthermore, ICP-MS is more sensitive than ICP-OES. However, both devices are used in corrosion studies with the appropriate sample preparation protocol.[1,2,8,13,14]

In our study, more Ni was released by orthodontic appliances with NiTi than with SS or CuNiTi archwires in the absence of *S. mutans*; this finding is consistent with that of Barrett et al.[2] However, some study findings are controversial. Contrary to the findings of Barrett et al., Karnam and Reddy[14] found no

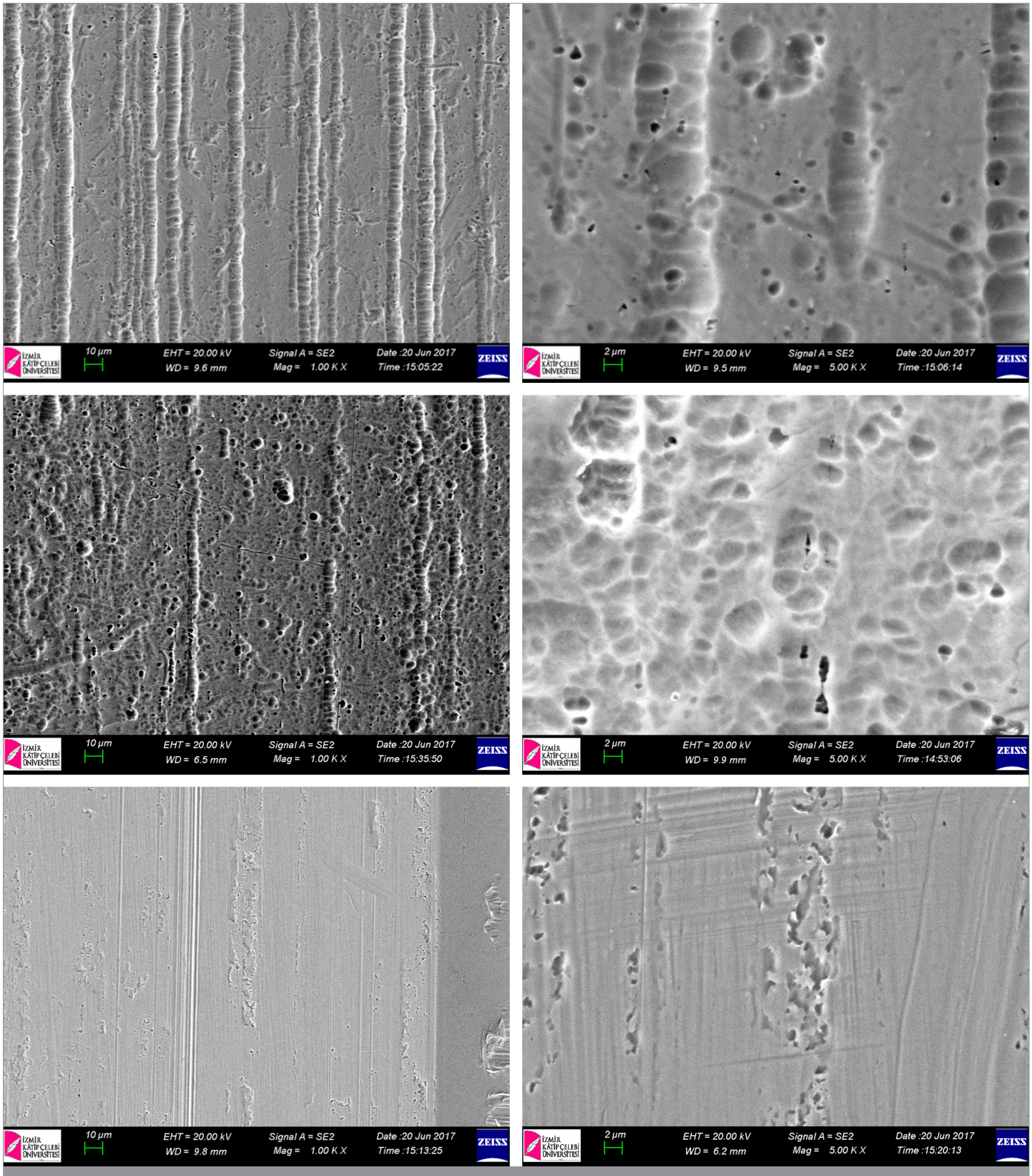

Figure 5. Scanning electron microscopy images of archwires in the control group 1 subgroups. Subgroups: CuNiTi (a, b), NiTi (c, d), and SS (e, f) archwires. Magnification: 1.00 KX (a, c, e) and 5.00 KX (b, d, f).

differences in the Ni-release rates of orthodontic appliances with SS, NiTi, CuNiTi or Elgiloy® archwires, and SS brackets. Moreover, Hwang et al.[1] reported that more Ni ion was released by SS than by CuNiTi or Sent alloy® and BioForce® archwires, and Kuhta et al.[13] found that SS archwires released the most Ni ions at pH 3.5 and 6.8 compared with thermal NiTi and NiTi archwires. The surface topography of an alloy is related to corrosion behavior.[15,16] In the present study, the surface roughness of the archwires was examined. Based on the findings of previous studies,[17,18] as well as our AFM and SEM findings, the surface of NiTi archwires is

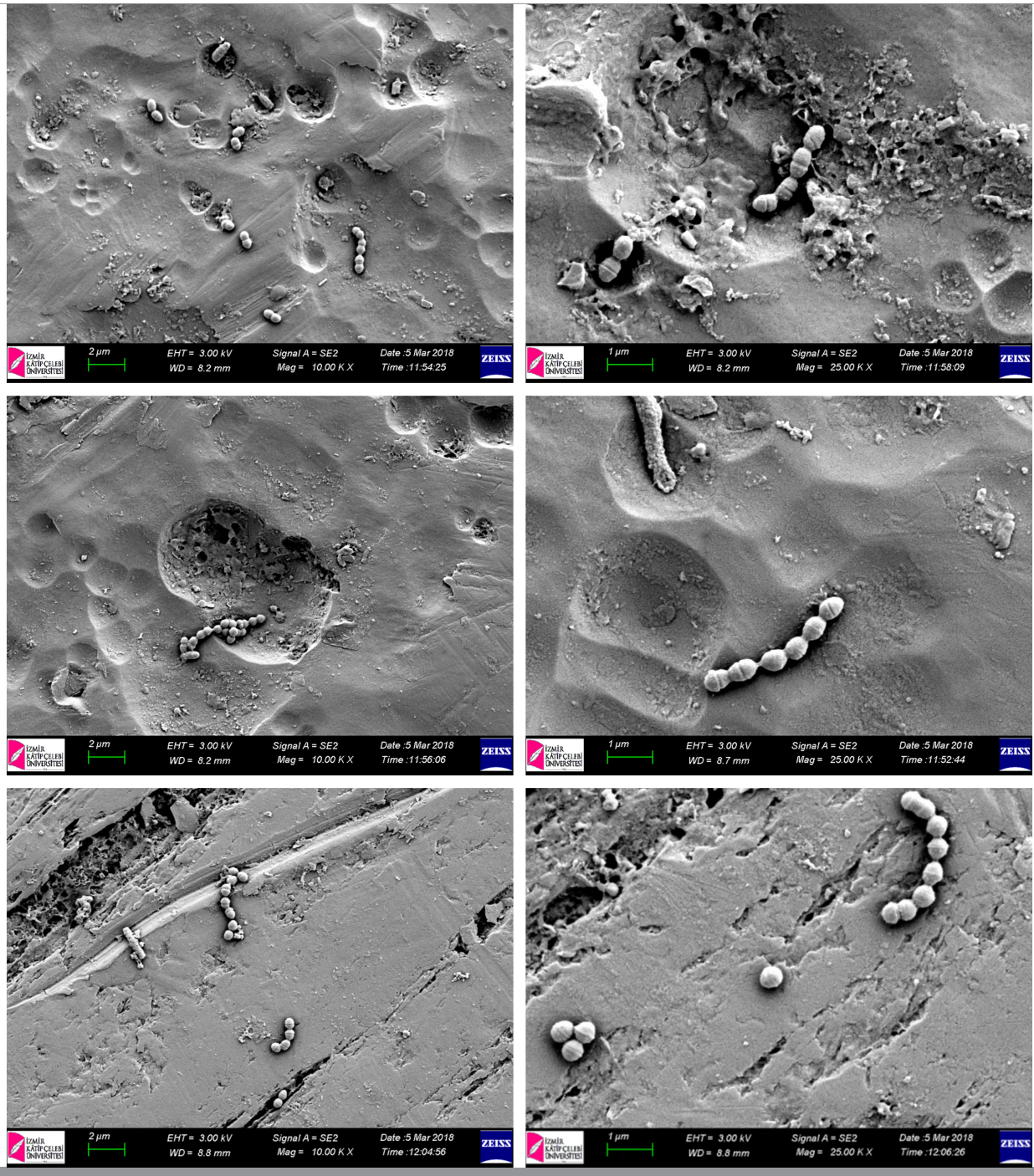

Figure 6. Scanning electron microscopy images of archwires with adherent *Streptococcus mutans*. *S. mutans* adhered to all types of archwires in the experimental group subgroups. Subgroups: CuNiTi (a, b), NiTi (c, d), and SS (e, f) archwires. Magnification: of 1.00 KX (a, c, e) and 5.00 KX (b ,d, f)

Table 3. Comparison of the counts of *Streptococcus mutans* in control group 2 and subgroups of the experimental group

	Initial	CG2	Group A_E	Group B_E	Group C_E	I/CG2 *P*	CG2/A *P*	CG2/B *P*	CG2/C *P*
cfu/mL	10^4	$10^6 \pm 5.1 \times 10^2$	$5 \times 10^4 \pm 5.19 \times 10^2$	$5.3 \times 10^4 \pm 1.1 \times 10^2$	$11 \times 10^4 \pm 8 \times 10^2$	.00*	.00*	.00*	.00*

*Significant difference at $P < .05$. CG 2, control group 2; E, experimental; I, initial density of inoculated *Streptococcus mutans* cells. Appliances with CuNiTi archwires, group A; appliances with NiTi archwires, group B; appliances with SS archwires, group C.

Table 4. Comparison of the counts of *S. mutans*

Groups	P
Initial/group A_E	1
Initial/group B_E	1
Initial/group C_E	0.78
Group A_E/group B_E 1	1
Group B_E/group C_E 1	1
Group AE/group C_E 1	1

Significant difference at P<0.05. I, initial density of inoculated *S. mutans*; E: Experimental. Appliances with CuNiTi archwires, Group A; appliances with NiTi archwires, Group B; appliances with SS archwires, Group C.

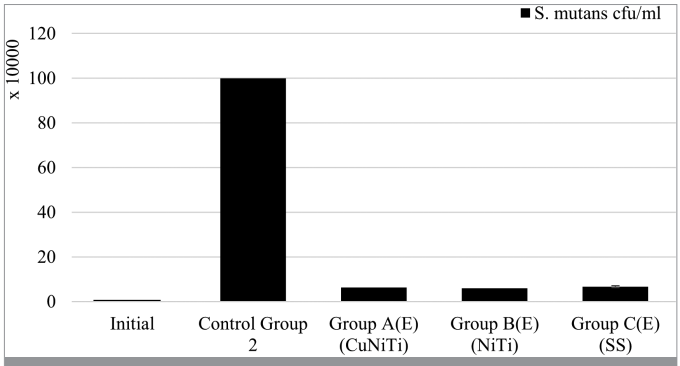

Figure 7. Density of *Streptococcus mutans* in control group 2 and the experimental group subgroups A_E, B_E, and C_E. I, original density of inoculated *S. mutans* cells

rougher than that of SS archwires. Increased surface roughness should be considered as a contributing factor to the corrosive behavior of orthodontic archwires by facilitating and increasing ion release.[19,20] Thus, it was considered that increased Ni-release from appliances with NiTi compared with that from appliances with SS archwires was associated with the rougher surface of NiTi than that of SS archwires.

The passive protective oxide layer on the surface of a metal or alloy provides resistance to corrosion and self-repair. Protective layers comprise Cr_2O_3 oxide on SS archwires and TiO_2 oxide on archwires that contain Ti. In alloys containing Ti, the layer of Ti-oxide is stronger than that of Cr-oxide, thus increasing corrosion resistance.[21] However, other factors may increase the corrosion rate of Ti-containing alloys. Alloys containing Ti have rougher surfaces than SS archwires, which can lead to the galvanic corrosion of these alloys.[22,23] Moreover, manufacturing defects in NiTi wires can be another factor that increase corrosion due to the rougher texture.[22] As many factors lead to corrosion, it was considered that various brands of alloys are produced using different methods or that differences in the surface topography of alloys and study designs may have led to contradictory results.

The corrosion current of NiTi archwires is higher than that of SS wires in electrochemical tests involving oral bacteria.[6,24,25] Kameda et al.[8] used ICP-OES to investigate the corrosive behavior of orthodontic appliances with SS and NiTi archwires in a high-risk caries environment inoculated with *S. mutans* and *Streptococcus sanguinis*. They reported that the surface of the SS appliances became rougher, and their ICP-OES results showed that Ni is released from SS, but not from Ni-Ti appliances, when cultured with oral bacteria. Similar to the findings of Kameda et al.[8] in this study, it was observed that *S. mutans* increased the surface roughness of SS archwires and caused Ni-release from orthodontic appliances with SS archwires. However, less Ni was released from orthodontic appliances with SS than from those with NiTi, probably due to the low abundance of *S. mutans*.

Biofilms produced on metal surfaces by *S. mutans* can lead to a localized acidic environment that promotes corrosion; therefore, the pH in an environment might not always reflect local pH changes.[8] In this study, a slight infection with *S. mutans* did not change the pH of artificial saliva with immersed appliances. Thus, the effects of *S. mutans* could be ascribed to localized changes in pH, and archwires made of SS might be more sensitive to the changes in localized pH than those made of NiTi. Our findings also suggest that while oral bacteria affect the corrosion of SS appliances, galvanic corrosion might occur primarily in Ni-Ti appliances.[22-23] Moreover, adding Cu to NiTi archwires increases the biocompatibility of NiTi archwires.[26] In the present study, it is found that CuNiTi wires released less Ni than NiTi wires in the artificial saliva with or without *S. mutans* although the Ra values of CuNiTi and NiTi archwires were similar.

An investigation of the effects of 16 pure metals on *S. mutans* growth in vitro showed that the corrosion process significantly depends on bacteriostasis[27] and that Co, CuNi, Ti, Fe, and vanadium inhibit the growth of the organism. Our findings of the effects of orthodontic alloys on the growth of *S. mutans* in artificial saliva in vitro were comparable with these results. Furthermore, it was found that *S. mutans* did not grow in the presence of corrosion.

Bacteria preferentially colonize rough surfaces over smooth surfaces.[28] The degree of surface roughness does not significantly affect bacterial adhesion after 6 hours of incubation with microorganisms.[29,30] Our SEM images were acquired after incubating the 3 types of archwire types with *S. mutans* for 3 days. Therefore, the rates of adhesion were similar even when the surface roughness of the archwires differed.

Many factors can affect corrosion, such as microbiological, enzymatic, ionic, and thermal properties in the oral environment. It is not possible to simulate the exact oral environment in vitro. However, it is important to determine the effect of the factors individually in an environment where many variables are present at the same time in order to understand the corrosion mechanism. For this reason, in vitro study is important. In this study, the density of *S. mutans* in an individual with good oral hygiene was considered. In order to understand the interaction between *S. mutans* and corrosion in a broader context, the effect of *S. mutans* on corrosion at different pH and different concentrations should be investigated.

CONCLUSION

The relationship between corrosion product formation and *S. mutans* is reciprocal, as corrosion inhibited the growth of *S. mutans* in Klimek artificial saliva. Even at a low density of *S. mutans*, Ni-release increased in appliances with SS archwires, indicating that *S. mutans* promotes corrosion. As rough surfaces can also promote corrosion, surface properties should be considered when evaluating the corrosion properties of any metal alloy.

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Informed Consent: Informed Consent is not applicable because this article does not contain any studies with human subjects.

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