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**Araştırma Makalesi / Research Article**

# Investigation of Nickel and Chromium Ion Release from Simulated Fixed Orthodontic Appliances in Artificial Saliva Containing Fluoride

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**ABSTRACT:** Fluoride is found in many oral hygiene products due to its anti-cariogenic effect. However, fluoride has a corrosive effect that begins at the time of application and continues with the fluoride remaining in the residual saliva. This study aimed to investigate the effect of different fluoride concentrations on the release of nickel and chromium ions from simulated fixed orthodontic appliances made of copper-nickel-titanium (Cu-NiTi), nickel-titanium (NiTi) and stainless steel (SS) archwires. Simulated orthodontic appliances in the experimental groups were immersed in Klimek artificial saliva containing 125, 500, or 900 ppm fluoride (NaF) for one hour and 0.1 ppm fluoride for eleven hours. The process was repeated in subsequent periods. Control groups were exposed to only Klimek artificial saliva. The amounts of nickel and chromium ions released into Klimek artificial saliva were measured using inductively coupled plasma mass spectroscopy (ICP-MS). The morphological characteristics of the archwires were examined using atomic force microscopy (AFM) and scanning electron microscopy (SEM). The average roughness value of SS archwires before and after immersion was lower than that of Cu-NiTi or NiTi archwires ($p<0.05$). Simulated orthodontic appliances with SS archwires released more chromium ions than simulated orthodontic appliances with Cu-NiTi and NiTi archwires in experimental groups with 125 ppm fluoride ($p<0.05$). Simulated fixed orthodontic appliances with SS archwires released fewer nickel ions than simulated fixed orthodontic appliances with Cu-NiTi archwires in both control and experimental groups ($p<0.05$). Additionally, simulated fixed orthodontic appliances with SS archwires released fewer nickel ions than those with NiTi archwires in control and experimental groups with 500 ppm fluoride ($p<0.05$).

**Keywords:** Fluoride effect, Corrosion, Cu-NiTi, NiTi, Stainless Steel, Archwire.

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## 1. INTRODUCTION

Stainless steel (SS) brackets, nickel-titanium (NiTi), copper-nickel-titanium (Cu-NiTi), and SS archwires are commonly used in fixed orthodontic treatments. Corrosion, defined as the deterioration of metals or metal alloys due to oxidation or other chemical processes (Brantley and Eliades, 2001), is an unavoidable occurrence in the oral environment, leading to the release of ions into the oral cavity (Eliades and Athanasiou, 2002). Nickel and chromium are the predominant ions released from alloys used in orthodontic treatments. The genotoxic, mutagenic, and cytotoxic effects of nickel and chromium ions can contribute to conditions such as contact allergies, asthma, hypersensitivity, and congenital disabilities (Genelhu et al., 2005; Mikulewicz and Chojnacka, 2010).

Fixed orthodontic treatment can lead to worsening of oral hygiene as brushing becomes more complicated and retention areas for plaque accumulation increase. For this reason, anti-cariogenic agents such as fluoride are frequently used in individuals receiving orthodontic treatment. However, fluoride has a corrosive effect. The passive protective oxide layer on metal or alloy surfaces ensures corrosion resistance and self-repair. The oxide layer in titanium-containing alloys is stronger than SS alloys (Schiff et al., 2002); however, fluoride ions can degrade both chromium oxide and titanium oxide layers (Huang, 2002; Nakagawa et al., 2002; Schiff et al., 2002; Huang, 2003; Huang and Lee, 2005; Heravi at al., 2015).

Fluoride-induced corrosion occurs during exposure and is then continued by residual fluoride in saliva. When fluoride products are used regularly, saliva is constantly contained in varying concentrations of fluoride. Baeshen et al. (2010) investigated salivary fluoride levels following the use of oral care products containing sodium fluoride for patients undergoing orthodontic treatment. They found that fluoride concentration in saliva ranged from 100 to 2200 ppm within one hour. When fluoride products are used regularly, saliva maintains therapeutic fluoride levels of around 0.1 ppm (ten Cate and Duijsters, 1983; Campus et al., 2003). Considering the corrosion-promoting effect of fluoride and the potential biological damage of increased corrosion, it is crucial to examine the corrosion behaviour of orthodontic appliances in the presence of fluoride. Consequently, this study aims to address this research gap by examining the corrosion behaviour of simulated orthodontic appliances with Cu-NiTi, NiTi, and SS archwires in the presence of fluoride while considering the fluoride concentration changes over time.

## 2. MATERIALS AND METHODS

### 2.1 Materials

Fixed orthodontic appliances consisted of 4 structurally identical lower incisor brackets, 3-cm-long (0.021 x 0.025-inch) Cu-NiTi, NiTi, or SS archwires tied with elastic ligatures (American Orthodontics Inc., Wisconsin, America). Artificial saliva solution was prepared according to the formula given by Klimek et al. (1982). Sodium fluoride (NaF) (Merck Darmstadt, Germany) was used as a fluoride source. The fluoride concentrations of 0.1 ppm, representing residual saliva (ten Cate and Duijsters, 1983; Campus et al., 2003) and 125, 500 and 900 ppm fluoride, representing application and one hour afterwards (Baeshen et al., 2010), were used. Klimek artificial saliva and simulated fixed orthodontic appliances were sterilised before the experiment.

### 2.2 Methods

This study was designed with nine experimental groups, each replicated three times, and one control group also replicated three times, and consisted of three periods of 12 (1+11) hours (36 hours in total). In the experimental groups, simulated fixed orthodontic appliances with Cu-NiTi, NiTi, or SS archwires were immersed in 3 mL of Klimek artificial saliva (pH 6.9) containing 125, 500, or 900 ppm sodium fluoride for one hour. Subsequently, the appliances were transferred to 3 mL of Klimek artificial saliva with 0.1 ppm fluoride for an additional 11 hours. The process was repeated in the second and third periods. The same protocol was applied to the control groups, which were only exposed to Klimek artificial saliva. To control the contamination, 100 μL of the liquid was inoculated into Brain Heart Infusion Agar following the transfer of simulated fixed orthodontic appliances to the new environment. The collected liquids were then transferred into containers based on their respective groups.

In this study, the amounts of nickel and chromium ions released into Klimek artificial saliva were determined by inductively coupled plasma mass spectroscopy (ICP-MS). Atomic force microscopy (AFM) and scanning electron microscopy (SEM) were used to analyse the morphological features of archwires before and after immersion. The schematic of the study is illustrated in Figure 1.

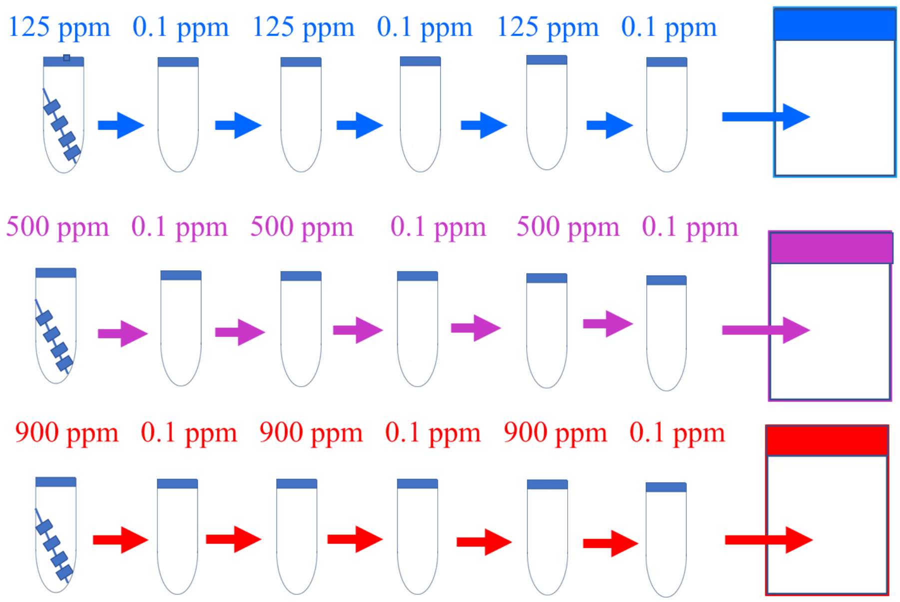


**Figure 1:** The schematic illustration of the study

### 2.3 Statistical Analysis

As the first step of the analysis, compliance with normal distribution was tested using the Shapiro-Wilk test. ANOVA test was used for comparisons between three groups regarding a normally distributed independent variable. Post Hoc Bonferroni analysis was conducted to reveal the group or groups that made the difference. An Independent Sample T-test was applied to compare two normally distributed groups. Independent Sample T-test was used when the assumptions were met, and the Whitney U test was used when they were not met.

## 3. RESULTS AND DISCUSSION

No growth was observed on the Brain Heart Agar, indicating the absence of contamination in the samples.

### 3.1 Composition of Archwires

The composition of archwires is shown in Table 1.

**Table 1:** Composition of archwires

| | Nickel | Titanium | Chromium | Copper | Iron |
|---|---|---|---|---|---|
| | % (w/w) | % (w/w) | % (w/w) | % (w/w) | % (w/w) |
| **Cu-NiTi** | 48.45 | 45.26 | 0.32 | 5.64 | 0.33 |
| **NiTi** | 54.28 | 44.59 | 0.10 | 0.62 | 0.41 |
| **SS** | 7.9 | 0.34 | 20.26 | 0.38 | 69.40 |

## 3.2 Ion Release (ICP/MS Analysis)

In this study, simulated fixed orthodontic appliances comprised structurally identical SS brackets with different orthodontic archwires. The variations in the types of orthodontic archwires are likely attributed to differences in the release of nickel and chromium ions. The comparison of nickel and chromium ion concentrations released in the control groups is presented in Table 2 and Figure 2. No significant difference was detected in the average amount of chromium ion release among the control groups. However, nickel ion was released the most in simulated orthodontic appliances with Cu-NiTi archwires, while the least amount was released in appliances with SS archwires (p=.000*, p=.000*, and p=.000*).

**Table 2:** Comparison of the concentrations of nickel and chromium ions released in control groups

| Ion | Group | Mean (ppb) | SD | Test Statistics | p | Bonferroni results | p |
|---|---|---|---|---|---|---|---|
| **Cr** | Cu-NiTi | 19.05 | 1.937 | 3.789** | .150 | | |
| | NiTi | 22.08 | 4.483 | | | | |
| | SS | 17.38 | 1.119 | | | | |
| **Ni** | Cu-NiTi | 25.38 | 2.535 | 75.385 | .000* | CuNiTi/NiTi | .000* |
| | NiTi | 18.87 | 1.660 | | | NiTi/SS | .000* |
| | SS | 12.74 | 0.587 | | | CuNiTi/SS | .000* |

*$p$<0.05. ANOVA, Kruskal Wallis**. Abbreviations: Cr: Chromium, Ni: Nickel, SD: Standard Deviation, Cu-NiTi: Simulated orthodontic appliances with Cu-NiTi archwires, NiTi: Simulated orthodontic appliances with NiTi archwires, SS: Simulated orthodontic appliances with SS archwires.

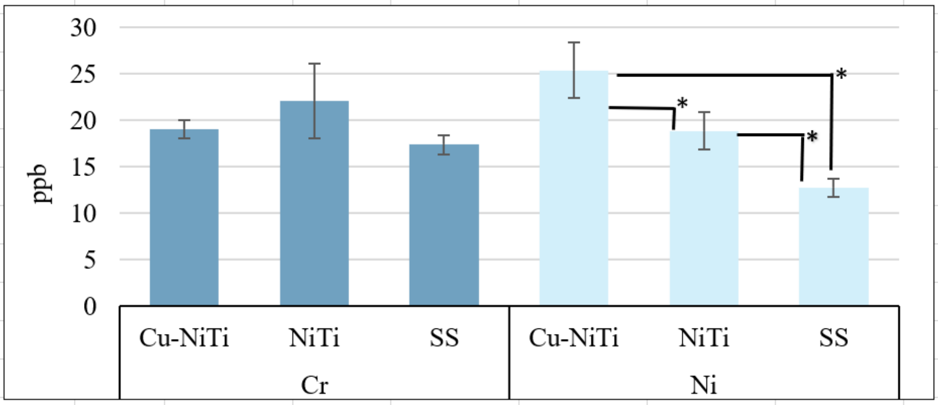


**Figure 2**: Comparison of the concentrations of nickel and chromium ions released in control groups. Abbreviations: *$p$<0.05, Cr: Chromium, Ni: Nickel, Cu-NiTi: Simulated orthodontic appliances with Cu-NiTi archwires, NiTi: Simulated orthodontic appliances with NiTi archwires, SS: Simulated orthodontic appliances with SS archwires.

Although titanium-containing archwires are considered more biocompatible than SS archwires, the results from various studies in the literature are contradictory (Barret et al., 1993; Hwang et al., 2000; Kuhta et al., 2009; Karnam and Reddy., 2012; Močnik et al., 2017; Chantarawaratit and Yanisarapan, 2021; Titiz et al., 2022). In line with the present study, our previous research also indicated a higher rate of nickel release in simulated orthodontic appliances with NiTi archwires than those with SS archwires (Titiz et al., 2022). Similarly, Močnik et al. (2017) reported that SS archwires released 32.6 μg/cm$^2$/week nickel, while NiTi archwires released 69.4 μg/cm$^2$/week nickel during simulated wear in the oral cavity. Consistent with our findings, Chantarawaratit and Yanisarapan (2021) reported that simulated orthodontic appliances with NiTi archwires released more nickel than those with SS archwires in neutral artificial saliva. However, in the related study, simulated orthodontic appliances with SS archwires released more chromium ions than those with NiTi archwires (Chantarawaratit and Yanisarapan, 2021). Similar to the current study, Karnam and Reddy (2012) and Barret et al. (1993) found no difference in chromium ion release in simulated orthodontic appliances with Cu-NiTi, NiTi, and SS archwires (Karnam and Reddy, 2012) or between SS and NiTi archwires (Barret et al., 1993). However, no difference in nickel release was also found between simulated orthodontic appliances in the related studies (Karnam and Reddy, 2012; Barret et al., 1993). Kuhta et al. (2009) and Hwang et al. (2000) reported that SS archwires released more nickel and chromium ions than NiTi archwires. Notably, in the study by Hwang et al. (2000), only SS archwires were heat treated in an electric furnace at 500 ºC for one minute and were then quenched in water. Heat treatment decreases the corrosion resistance of alloys, potentially leading to increased ion release from SS archwires.

In the experimental groups containing 125 ppm fluoride, simulated orthodontic appliances with SS archwires released more chromium ions than those with Cu-NiTi and NiTi archwires (*p*=.002 and *p*=.001) (Table 3, Figure 3). Moreover, in groups containing 125, 500 and 900 ppm fluoride, simulated orthodontic appliances with Cu-NiTi archwires released more nickel ions than simulated orthodontic appliances with SS archwires (*p*=.002, *p*=.002, *p*=.003). Additionally, in the groups containing 500 ppm fluoride, simulated orthodontic appliances with NiTi archwires released more nickel ions than those with SS archwires (*p*=.002) (Table 3, Figure 3).

**Table 3:** Comparison of the amounts of nickel and chromium ions released in the experimental groups

| Fluoride | Ion | Group | Mean (ppb) | SD | Test Statistics | p | Bonferroni results | p |
|---|---|---|---|---|---|---|---|---|
| 125 ppm | Cr | Cu-NiTi | 18.95 | 0.148 | 15.041 | .000* | CuNiTi/SS | .002* |
| | | NiTi | 18.52 | 0.58 | | | NiTi/SS | .001* |
| | | SS | 23.60 | 2.88 | | | | |
| | Ni | Cu-NiTi | 19.18 | 0.54 | 8.096 | .004* | CuNiTi/SS | .003* |
| | | NiTi | 17.43 | 1.92 | | | | |
| | | SS | 16.05 | 1.21 | | | | |
| 500 ppm | Cr | Cu-NiTi | 19.77 | 1.32 | 1.004 | .390 | | |
| | | NiTi | 20.60 | 0.90 | | | | |
| | | SS | 20.98 | 2.07 | | | | |
| | Ni | Cu-NiTi | 23.21 | 0.69 | 23.937 | .000* | CuNiTi/SS | .002* |
| | | NiTi | 20.36 | 3.57 | | | NiTi/SS | .002* |
| | | SS | 14.77 | 0.75 | | | | |
| 900 ppm | Cr | Cu-NiTi | 16.48 | 1.32 | 1.354 | .292 | | |
| | | NiTi | 16.23 | 0.36 | | | | |
| | | SS | 17.09 | 0.16 | | | | |
| | Ni | Cu-NiTi | 47.08 | 28.32 | 11.882** | .003* | Cu-NiTi/SS | .002* |
| | | NiTi | 18.76 | 3.09 | | | | |
| | | SS | 14.95 | 0.17 | | | | |

*$p$<0.05. ANOVA, Kruskal Wallis** Abbreviations: Cr: Chromium, Ni: Nickel, 125 ppm: Experimental groups containing 125 ppm fluoride, 500 ppm: Experimental groups containing 500 ppm fluoride, 900 ppm: Experimental groups containing 900 ppm fluoride, Cu-NiTi: orthodontic appliances with Cu-NiTi archwires, NiTi: Simulated orthodontic appliances with NiTi archwires, SS: Simulated orthodontic appliances with SS archwires

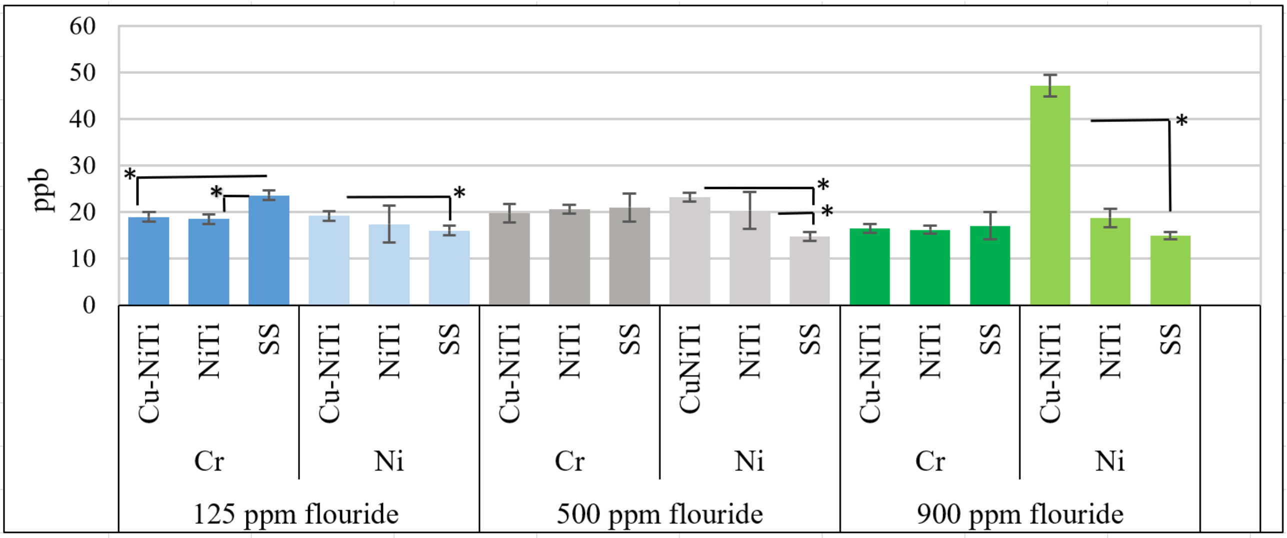


**Figure 3:** Nickel and chromium release in Experimental Groups at different fluoride concentrations. $*p<0.05$ Abbreviations: Cr: Chromium, Ni: Nickel, 125 ppm: Experimental groups containing 125 ppm fluoride, 500 ppm: Experimental groups containing 500 ppm fluoride, 900 ppm: Experimental groups containing 900 ppm fluoride, Cu-NiTi: Simulated orthodontic appliances with Cu-NiTi archwires, NiTi: Simulated orthodontic appliances with NiTi archwires, SS: Simulated orthodontic appliances with SS archwires

To the best of our knowledge, relatively few studies have specifically investigated the corrosion behaviours of Cu-NiTi, NiTi, and SS archwires in the presence of sodium fluoride using immersion tests. Chantarawaratit and Yanisarapan (2021) and Yanisarapan et al. (2018) reported that simulated orthodontic appliances with NiTi archwires released more nickel ions than those with SS archwires in artificial saliva containing 1000 ppm sodium fluoride. Similarly, Condò et al. (2022) found that SS archwires released less nickel but more chromium ions after 168 hours of immersion in neutral artificial saliva containing 400 ppm sodium fluoride. Močnik et al. (2017) investigated the effects of fluoride (600 ppm, 1000 ppm, 3200 ppm) on corrosion using electrochemical tests in artificial saliva. They reported that the SS alloy exhibited better properties at high fluoride concentrations than the NiTi alloy. Pulikkottil et al. (2016) found that the corrosion resistance of NiTi archwires was higher than that of the SS archwires in an environment containing 2250 ppm sodium fluoride (pH 6.5). Similarly, Heravi et al. (2014) determined that the corrosion resistance of NiTi archwires was higher than SS archwires in the presence of 500 ppm and 2000 ppm sodium fluoride (pH 5.3).

The resistance of metals or alloys to corrosion can be determined through immersion tests, which measure the number of ions passing into the liquid, or electrochemical tests. However, corrosion resistances determined by electrochemical tests may not fully reflect the conditions in the oral environment. Therefore, immersion tests are more reliable for examining the corrosion behaviour of metals in the oral environment (Luft et al., 2009). While SS wires have demonstrated lower corrosion resistance in electrochemical tests (Pulikkottil et al., 2016; Heravi et al., 2014), numerous studies suggest that SS wires exhibit greater biocompatibility when considering nickel release in artificial saliva containing fluoride (Močnik et al., 2017; Yanisarapan et al., 2018; Chantarawaratit and Yanisarapan, 2021; Condò et al., 2022).

### 3.3 Surface Roughness

A statistically significant difference was detected in the average roughness (Ra) of Cu-NiTi, NiTi, and SS as-received archwires (Table 4 and Figure 4). The Ra value of NiTi and Cu-NiTi as-received archwires was higher than that of the as-received SS archwires. The Cu-NiTi archwire's Ra value was higher than the SS archwires in the control groups. The Ra values of Cu-NiTi and NiTi archwires were higher than SS archwires in all experimental groups (125, 500 and 900 ppm). AFM surface morphologies of archwires, both as-received and after immersion, are presented in Figure 5.

**Table 4:** Comparison of the average roughness values of archwires

| Group | Archwire | Mean (nm) | SD | Test Statistic | p | Bonferroni result | p |
|---|---|---|---|---|---|---|---|
| **As-received** | Cu-NiTi | 32.06 | 6.34 | 27.389 | <0.001* | Cu-NiTi/NiTi | 1.000 |
| | NiTi | 35.94 | 8.30 | 2.937 | 0.027* | Cu-NiTi/SS | 0.05* |
| | SS | 6.28 | 2.39 | 1.893 | 0.076 | NiTi/SS | 0.05* |
| **Control group** | Cu-NiTi | 55.75 | 15.85 | | | Cu-NiTi/NiTi | 0.433 |
| | NiTi | 33.18 | 5.65 | | | Cu-NiTi/SS | 0.048* |
| | SS | 19.72 | 18.98 | | | NiTi/SS | 1.000 |
| **125 ppm fluoride** | Cu-NiTi | 61.66 | 16.33 | | | Cu-NiTi/NiTi | 1.000 |
| | NiTi | 75.72 | 25.30 | | | Cu-NiTi/SS | 0.002* |
| | SS | 9.02 | 6.10 | | | NiTi/SS | <0.001* |
| **500 ppm fluoride** | Cu-NiTi | 67.69 | 15.59 | | | Cu-NiTi/NiTi | 1.000 |
| | NiTi | 89.55 | 74.28 | | | Cu-NiTi/SS | 0.002* |
| | SS | 6.12 | 1.37 | | | NiTi/SS | 0.031* |
| **900 ppm fluoride** | Cu-NiTi | 58.36 | 22.41 | | | Cu-NiTi/NiTi | 1.000 |
| | NiTi | 45.00 | 15.71 | | | Cu-NiTi/SS | 0.002* |
| | SS | 4.58 | 0.98 | | | NiTi/SS | 0.031* |

*p<0.05. Two-Way ANOVA. Abbreviations: SD: Standard Deviation, 125 ppm: Experimental groups containing 125 ppm fluoride, 500 ppm: Experimental groups containing 500 ppm fluoride, 900 ppm: Experimental groups containing 900 ppm fluoride.

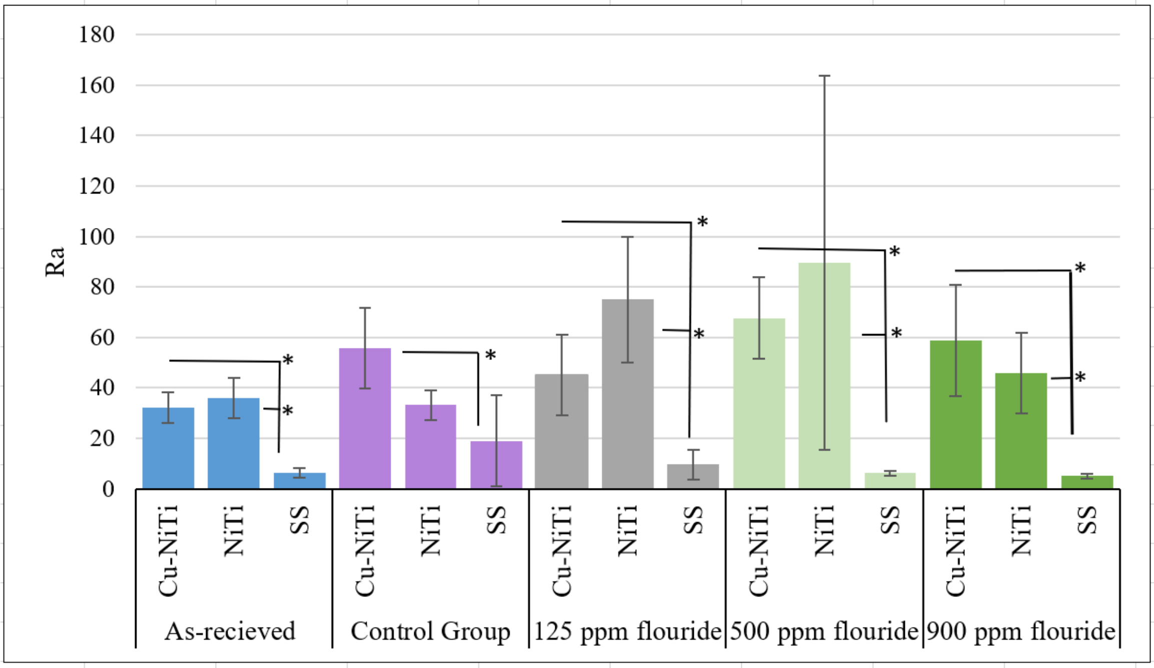


**Figure 4:** Comparison of the Ra (in nm) values of as-received and post-immersed archwires. *$p$<0.05. Two-Way ANOVA. Abbreviations: 125 ppm: Experimental groups containing 125 ppm fluoride, 500 ppm: Experimental groups containing 500 ppm fluoride, 900 ppm: Experimental groups containing 900 ppm fluoride.


**Figure 5:** Atomic force microscopy (AFM) surface morphologies of as-received and post-immersed archwires Abbreviations:125 ppm: Experimental groups containing 125 ppm fluoride, 500 ppm: Experimental groups containing 500 ppm fluoride, 900 ppm: Experimental groups containing 900 ppm fluoride Each row belongs to the same group.

### 3.4 SEM Results

Figure 6 displays SEM images, revealing that the SS archwires appeared smoother than the NiTi and Cu-NiTi archwires, both as-received and post-immersed states. These SEM observations align with the Ra values from AFM.

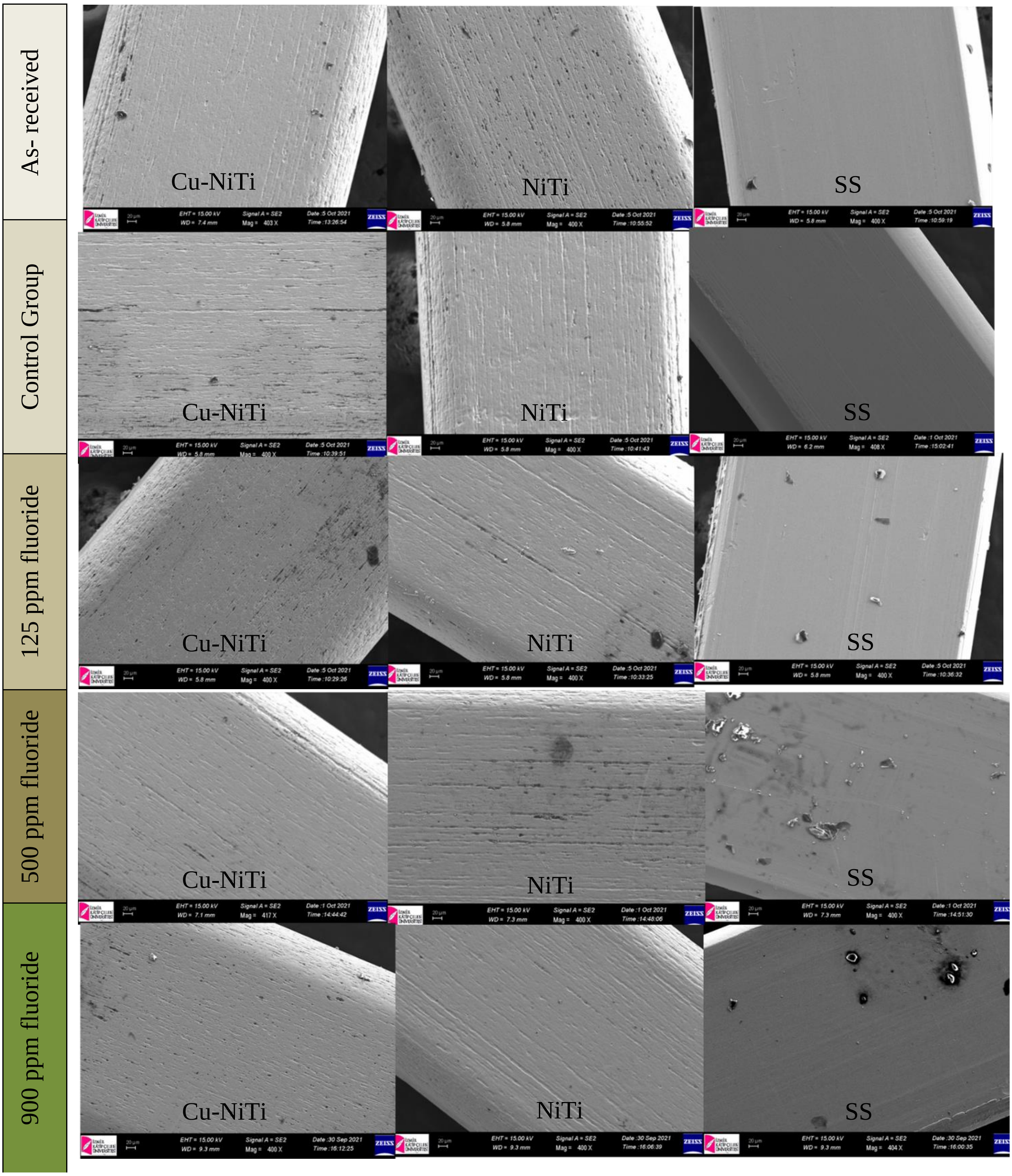


**Figure 6:** SEM photographs of as-received and post-immersed archwires. Abbreviations:125 ppm: Experimental groups containing 125 ppm fluoride, 500 ppm: Experimental groups containing 500 ppm fluoride, 900 ppm: Experimental groups containing 900 ppm fluoride Each row belongs to the same group.

The passive protective layer present on SS archwires ($Cr_2O_3$) and titanium-containing archwires ($TiO_2$) resists corrosion and self-healing. Since the $TiO_2$ layer is stronger than the $Cr_2O_3$ layer in Ti-containing alloys, alloys containing titanium are expected to exhibit greater durability compared to stainless steel (Schiff et al., 2002). However, it's important to note that other factors can influence the corrosion rate of titanium-containing alloys. In the current study, the SEM images and Ra results indicated that the surface roughness of SS archwires was lower than that of NiTi or Cu-NiTi archwires. The smoother surface state of SS archwires continued in both artificial saliva alone and artificial saliva containing various concentrations of fluoride. Archwires containing titanium exhibited rougher surfaces compared to SS archwires, which may contribute to the galvanic corrosion of these alloys (Oshida et al., 1992; Jacobs al., 1998). Additionally, manufacturing defects in NiTi archwires may be another factor in exhilarating corrosion (Oshida et al., 1992). Thus, surface roughness should be considered a significant factor contributing to the corrosive behaviour of orthodontic archwires, alongside the composition of protective oxide layers and other environmental factors.

During orthodontic treatment, the corrosion process is multifactorial and is affected by many factors, such as temperature, food acidity, microorganisms, and friction. To understand the corrosion process, it is important to search for the effects of the factors one by one, but the corrosion behaviour of alloys may change with the influence of other factors. For this reason, more comprehensive *in vitro* corrosion studies should be carried out, to understand the corrosion process of alloys considering the environment's temperature change, microbial flora, friction, and pH levels with fluoride.

## 4. CONCLUSION

This study was carried out to investigate the effect of fluoride on nickel and chromium release in a simulated intraoral environment. We concluded the following.

1. No significant difference was detected in the average amount of chromium ion release among the control groups.
2. Nickel ion was released the most in simulated orthodontic appliances with Cu-NiTi archwires, while the least amount was released in appliances with SS archwires in control groups.
3. In the experimental groups containing 125 ppm fluoride, simulated orthodontic appliances with SS archwires released more chromium ions than those with Cu-NiTi and NiTi archwires.
4. In experimental groups containing 125, 500, and 900 ppm fluoride, simulated orthodontic appliances with Cu-NiTi archwires released more nickel ions than those with SS archwires.
5. Simulated orthodontic appliances with NiTi archwires released more nickel ions than those with SS archwires in experimental groups containing 500 ppm fluoride.
6. No significant difference was observed in chromium and nickel release between simulated orthodontic appliances with CuNiTi or NiTi archwires in experimental groups containing 125, 500 and 900 ppm fluoride.
7. Surface roughness should be considered a significant factor contributing to the corrosive behaviour of orthodontic archwires.
8. The use of materials from different brands and testing with varying methods may reduce the comparability of corrosion studies in the literature.

## 5. ACKNOWLEDGEMENTS

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## 6. CONFLICT OF INTEREST

Authors approve that. to the best of their knowledge. There is no conflict of interest or common interest with an institution/organisation or a person that may affect the paper's review process.

## 7. AUTHOR CONTRIBUTION

Conceptualisation. Serap Titiz YURDAKAL; Methodology. Serap Titiz YURDAKAL and Safiye Elif KORCAN; Formal analysis Serap Titiz YURDAKAL and Safiye Elif KORCAN. Investigation. Serap Titiz YURDAKAL; Data curation. Serap Titiz YURDAKAL and Elif Esin HAMEŞ; Writing-original draft and review & editing. Serap Titiz YURDAKAL, Elif Esin HAMEŞ, and Atıf KOCA

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