Original Article

Cytotoxicity of Orthodontic Wire Corroded in Fluoride Solution In Vitro

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ABSTRACT

Objective: To investigate the toxicity of fluoride corrosion extracts of stainless steel (SS) and nickel-titanium (NiTi) wires on a human osteosarcoma cell line (U2OS).

Materials and Methods: The SS and NiTi wires were corroded by an electrochemical method with the application of three kinds of electrolytes: 0.2% pH 3.5 acidulated phosphate fluoride (NaF) in artificial saliva, and pH 4 and pH 6.75 artificial saliva solutions. The extracts were analyzed for nickel, chromium, and titanium ions by the atomic absorption method. The extracts were diluted with medium to different concentrations (1, 0.1, and 0.01 μL/mL). The cell survival rate was determined by the ability of test cells to cleave the tetrazolium salt to form a formazan dye.

Results: The results were compared using one-way analysis of variance. Differences between the treatment means were analyzed using a Student-Newman-Keuls (SNK) test and were considered significant at $P < .05$. The release of ionic nickel was different in different extract groups ($P < .05$). The SS and NiTi wires in the 0.2% pH 3.5 NaF artificial saliva group caused a dose-dependent decrease in the survival rate ($P < .05$). Survival rates of cells in the groups exposed to extracts of SS and NiTi wires in pH 4 and pH 6.75 artificial saliva solutions showed no statistical differences ($P > .05$).

Conclusions: Orthodontic wires in acidulated fluoride saliva solution can cause U2OS cell toxicity.

KEY WORDS: Stainless steel wire; Nickel titanium wire; Fluoride; Cytotoxicity

INTRODUCTION

The orthodontic metal wire has progressed from nickel-chromium-iron (Ni-Cr-Fe) stainless steel (SS) alloy to nickel-titanium (NiTi) alloy. This is related to its properties: (1) superelasticity, (2) thermal shape memory, (3) good corrosion resistance, and (4) good biocompatibility. In clinical orthodontic treatment, different metal alloys have a wide range of applications. The characteristics of NiTi wire alloy can reduce chair time and shorten treatment times.[1]

To achieve good oral hygiene care, there is an increasing use of dental gels and resins containing fluoride applied to prevent dental caries. The fluoride level in the oral cavity varies according to the prophylactic treatment.[2,3] Because the oral environment is favorable for the biodegradation of metal because of its ionic, thermal, microbiological, and enzymatic properties, it can be presumed that patients are exposed, to a certain extent, to the products of the corrosion processes.[4] A number of studies have demonstrated that metal ions can be released from metallic materials as the result of corrosion.[5–9] The corrosion phenomenon not only may influence the mechanical properties of the metal appliances, but also may affect the body after the metal ions have been released.[10–12]

Nickel (Ni) is one of the most common causes of allergic contact dermatitis, and the incidence of such contact dermatitis is as high as approximately 20–30%.[13–15] Adverse reactions related to Ni-containing orthodontic devices such as arch wires, brackets, and buckles on headgear devices have been observed.[16–18] Particularly interesting were two clinical studies that

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claimed that the use of NiTi arch wires can convert Ni-nonsensitive subjects into Ni-sensitive subjects, with an approximately 20% conversion rate.[19,20]

The corrosion behavior of orthodontic wires shows high corrosion resistance in various solutions, such as Ringer's solution,[21] artificial saliva,[22] and sodium chloride solution.[23] In these solutions, the corrosion resistance of titanium (Ti) alloys is higher than that of SS or cobalt-based alloys from the viewpoint of film breakdown. SS alloys are easily corroded in chloride-containing solutions, and Ti-containing alloys are easily corroded in acidified fluoride media.[11,24] Immersion in the fluoride solution will lead to degradation of the mechanical properties of NiTi wire because of hydrogen embrittlement.[12] One reason for this is that the breakdown of the protective oxide film leads to a decrease in corrosion resistance.[25,26]

According to atomic absorption studies, the concentration of ions released into media without cells present was not proportional to the composition of the alloys.[27] In order to study material toxicity of cells, it is suggested that using ionic release from alloys can provide a better understanding of cellular response.[28] In the present study, we used whole metal extracts to detect the cytotoxicity.

The purpose of the present study was to evaluate the toxicity of cell treated with different extracts from orthodontic wires corroded by electrochemical method in acidified phosphate fluoride (NaF) solutions.

MATERIALS AND METHODS

Wire Preparation and Potentiodynamic Control of Wire Corrosion

Two types of wire, SS (Fe-18Cr-8Ni) and heated activated Nitinol (NiTi) wires, were used. The sizes of the SS wires were 0.010 inches (0.25 mm), 0.014 inches (0.36 mm), and 0.016 $\times$ 0.022 inches (0.41 $\times$ 0.56 mm). The sizes of the NiTi wires were 0.016 inches (0.41 mm) and 0.016 $\times$ 0.022 inches (0.41 $\times$ 0.56 mm) (3M Unitek, Monrovia, Calif). Each test sample contained five pieces of wire of 5 mm in length.

The corrosive wire extracts were collected using an electrochemical method. The electrochemical corrosive breakdown of SS and NiTi wires was initiated by applying a potential higher than the predicted breakdown potential. The method followed the description of Shih et al[29] and three electrolytes were used in the corrosive reaction. First, artificial saliva (Table 1) containing 0.2% acidulated NaF (0.2 mass% NaF + 0.17 mass% H_3PO_4, pH 3.5) was used as the electrochemical corrosive electrolyte. To keep the acid condition, the electrolyte was adjusted to pH 3.5 using lactic acid and was maintained at 37°C. Second and third, the electrochemical corrosive electrolytes were adjusted in artificial saliva to pH 4 and pH 6.75. A potentiodynamic polarization machine was applied from −800 mV in the anodic direction with a scan rate of 1 mV/s after dipping the specimen into the electrolyte for 1 hour. After that, each corrosive electrolyte was collected and diluted in McCoy's medium (Sigma Chemical, St Louis, Mo) to different concentrations (1, 0.1, and 0.01 μL/mL) as the experimental groups. The control group used artificial saliva with the pH adjusted to 6.75.

Table 1. Contents of the Artificial Saliva[a]

Ingredient	Amount (mg)
Sodium chloride	0.844
Potassium chloride	1.2
Calcium chloride anhydrous	0.146
Magnesium chloride 6 H_2O	0.052
Potassium phosphate dibasic	0.34
Sorbitol solution 70%	60
Methyl paraben	2
Hydroxyethyl cellulose	3.5

[a] Sali Lube saliva substitute (Sinphar Pharm, Taipei, Taiwan).

Metal Ion Release Analyses

The method followed that used in our previous study.[30] A brief description follows: solutions of electrochemical corrosive electrolyte were added to polypropylene tubes containing SS wire or NiTi wire. The solution was analyzed for Ni, chromium (Cr), and Ti ions. Standards were prepared in equivalent solutions to counteract any buffer effects. The pretreatment and atomization temperatures recommended by Perkin-Elmer were used in the furnace programs to ensure that linear standard curves were obtained for each element. Each sample was analyzed for all three ions and concentrations, measured as μg/cm^2, averaged across the five replicates.

Results were compared using one-way analysis of variance. Differences in treatment means were analyzed using the Student-Newman-Keuls test and were considered to be significant at $P < .05$.

Cell Culture and Survival Test

The study for the biocompatibility of wires followed the method described by Schweikl and Schmalz[31] and Wataha et al.[32] Single-cell suspensions of a human osteosarcoma cell line (U2OS, Food Industry Research and Development Institute, Hsinchu, Taiwan) were seeded in 96-well flat-bottomed tissue culture plates at 5 $\times$ 10^3 cells per well, as determined by prior hemocytometer counting, in complete McCoy's medium, and incubated in a humidified atmosphere of 5% CO_2 in air at 37°C for 24 hours. The culture medium was then replaced with 200 μg/L aliquots of either the experimental groups or the control group, and cells

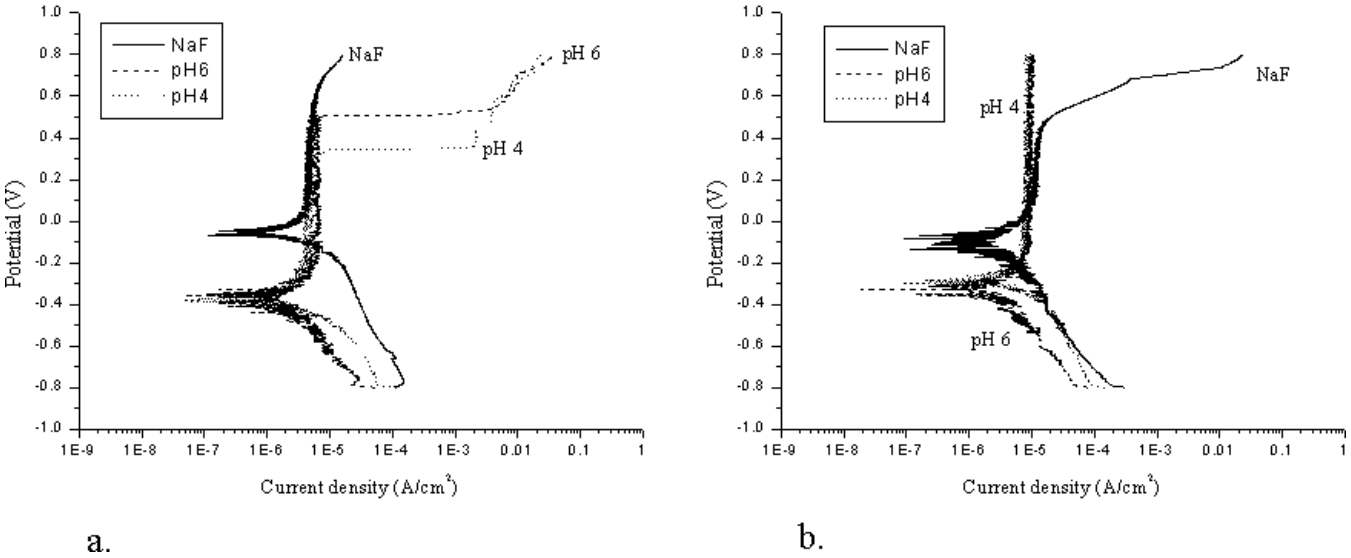

Figure 1. Cyclic potentiodynamic polarization curves of wires in different media.

were incubated for 24 hours at 37°C in a humidified 5% CO_2 in air environment.

Four wells were used for each concentration. Following exposure, the cell viability was determined by a technique that measures the ability of test cells to cleave the tetrazolium salt (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium bromide [MTT]) to a formazan dye. The medium was removed with a sterile pipette, and 200 μL of phosphate-buffered saline was added to each well, swirled gently for 1 min, and then replaced with 100 μL of complete medium and 10 μL of a 5 mg/L solution of MTT. The cells were incubated in the MTT/medium solution for 4 hours at 37°C in a 5% CO_2 in air atmosphere. One hundred microliters of a 6.25% vol/vol 0.1 mol/L NaOH in dimethyl sulfoxide solution was added to each well, following which the plates were incubated overnight to solubilize any formazan crystals that had formed. Plates were shaken for 60 minutes at room temperature in order to achieve uniformity of color of the well contents. Optical densities were then measured at a wavelength of 570 nm in a spectrophotometer. The survival rate was calculated as: survival % = (absorbance of the treated sample/absorbance of the medium) × 100%. The results were compared using one-way analysis of variance. Differences between the treatment means were analyzed using Student-Newman-Keuls test and were considered to be statistically significant at $P < .05$.

RESULTS

Corrosive Reactions of the Wires

The polarization curves of the SS and NiTi wires in different media are shown in Figure 1a, b. All specimens showed active-to-passive transition behavior.

Corrosive Metal Ions

The results of atomic absorption analysis of different mediums are presented in Table 2. The release of ionic Ni showed statistically significant differences in all groups ($P < .05$). In pH 6.75 artificial saliva medium, the release of Ni ions was higher from the SS than from the NiTi wire ($P < .05$). In pH4 artificial saliva medium and pH 3.5 NaF medium, the release of Ni ions was higher from the NiTi than from the SS wire ($P < .05$). Analysis of released Cr ions revealed a difference only in the pH 3.5 NaF medium group ($P < .05$). Analysis of released Ti ions revealed a difference only in the pH 3.5 NaF medium group ($P < .05$).

Survival Rates of Cells Exposed to Extracts of SS Wire

The survival rate of the group exposed to SS wire extracts showed statistically significant differences ($P < .05$; Figure 2C, F, I). The pH 3.5 NaF group showed a lower survival rate than that did the control group. In the pH 4 and pH 6.75 artificial saliva groups, survival rates did not show a statistically significant difference ($P > .05$).

Survival Rate of Cells Exposed to Extracts of NiTi Wire

Survival rates of cells exposed to extracts of NiTi wire showed statistically significant differences ($P < .05$) in the pH 3.5 NaF group (Figure 3C, F) and in the pH 6.75 artificial saliva group in which 0.016 × 0.022-inch NiTi wire was immersed (Figure 3A). In the pH 3.5 NaF group, the survival rates of the experimental groups were lower than that of the control group (Fig-

Table 2. Metal Ion (Nickel [Ni], Chromium [Cr], and Titanium [Ti]) Release Analyses from Fluoride-corroded Orthodontic Metal Wires. Five Replicates were Performed in Each Test. Tests were Performed in pH 6.75 Artificial Saliva (AS) Medium, pH 4 AS Medium, and pH 3.5 Fluoride (APF) Medium[a]

	Ni ($\mu g/cm^2$)	Cr ($\mu g/cm^2$)	Ti ($\mu g/cm^2$)
	Mean ± SD	Mean ± SD	Mean ± SD
A. pH 6.75 AS extracts			
0.016-inch NiTi	18.27 ± 3.28	—	0
0.016 × 0.022-inch NiTi	20.73 ± 5.43	—	0
0.014-inch SS	21.38 ± 5.32	12.63 ± 5.27	—
0.016 × 0.022-inch SS	26.21 ± 5.43	11.27 ± 4.47	—
0.010 inch SS	38.24 ± 8.35	15.21 ± 5.48	—
Control	0.28 ± 0.15	0.24 ± 0.13	—
P	.000	.484	—
B. pH 4 AS extracts			
0.016-inch NiTi	58.21 ± 4.37	—	21.42 ± 8.32
0.016 × 0.022-inch NiTi	69.27 ± 5.49	—	26.73 ± 9.28
0.014-inch SS	45.32 ± 6.43	17.28 ± 9.20	0
0.016 × 0.022-inch SS	50.48 ± 8.39	21.20 ± 11.27	0
0.010-inch SS	43.93 ± 5.68	29.51 ± 10.63	0
Control	0.28 ± 0.15	0.24 ± 0.13	—
P	.000	.207	.369
C. pH 3.5 NaF extracts			
0.016-inch Niti	67.28 ± 7.39	—	53.39 ± 4.56
0.016 × 0.022-inch NiTi	80.20 ± 8.37	—	67.83 ± 8.47
0.014-inch SS	68.38 ± 7.35	43.39 ± 6.30	—
0.016 × 0.022-inch SS	64.39 ± 8.82	37.71 ± 4.38	—
0.010 inch SS	64.54 ± 7.38	49.37 ± 6.49	—
Control	0.28 ± 0.15	0.24 ± 0.13	—
P	.028	.026	.01

[a] NiTi indicates nickel-titanium; SS, stainless steel.

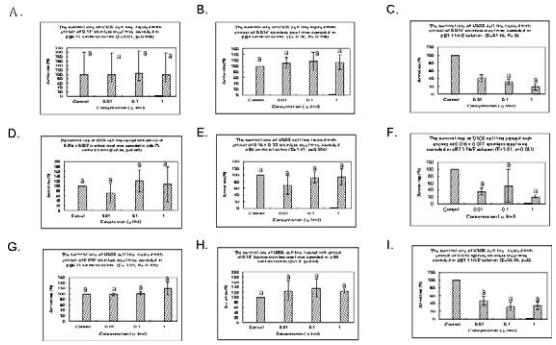

Figure 2. Survival rates (%) of U2OS cells treated with SS wire corrosion extracts. Extracts of SS wire were obtained by subjecting the wires to 0.2% NaF, pH 4.0 artificial saliva, and pH 6.75 artificial saliva. SNK rankings with the same letters do not significantly differ at $P < 0.05$.

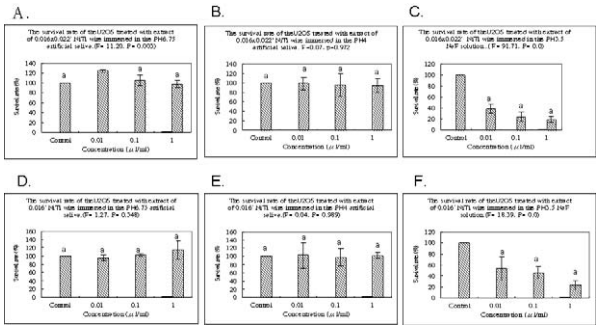

Figure 3. Survival rates of U2OS cells treated with NiTi wire corrosion extracts. Extracts of NiTi wire were obtained by subjecting the wires to 0.2% NaF, pH 4.0 artificial saliva, and pH 6.75 artificial saliva. SNK rankings with the same letters do not significantly differ at $P < 0.05$.

ure 3C, F). In the pH 6.75 artificial saliva and 0.016 × 0.022-inch NiTi wire group, the survival rate of 0.01 μL/mL concentration was higher than that of the control group. The remaining experimental groups (Figure 3B, D, E) showed no significant differences in the survival rate evaluation.

DISCUSSION

The corrosive status of the experimental wires was created through electrochemical means and was intended to increase corrosion speed compared to traditional immersive corrosion methods.[29] The present results showed that U2OS cells treated with extracts of SS corrosion appeared to have significant lower

survival rates in the 0.2% NaF, pH 3.5 solution (Figure 2). This low survival rate might be attributable to the following reasons. First, SS alloys are easily corroded in the presence of chloride ions.[33] In the present study, the artificial saliva contained chloride and the release of Ni ions was higher from the pH3.5 NaF group than from the other groups. Second, the electrochemical corrosion of 316 SS in acid media containing fluoride ions has been demonstrated.[34] It was shown that a low concentration of NaF ($<$0.001 M) had no significant influence on the passive performance of 316 SS. But a high concentration of NaF (0.1 M) markedly reduced the passive performance, and gave rise to a current peak below the pitting potential in 0.1 M NaF solutions.[33]

In the present study, the survival rate of U2OS cells decreased as the extracts concentration of SS in the 0.2% NaF increased. This indicates that the extracts of SS in the 0.2% NaF, pH 3.5 group contained more toxic material than did the extracts of SS in other solutions. For metal ion release, analysis of extracts showed higher Ni ion concentrations in the 0.2% NaF, pH 3.5 group than in the other groups.

Several studies have found Ni ions to be toxic as well as carcinogenic to cultured cells.[35–37] In the present study, some of the results showed that the survival rate of U2OS cells was higher in experimental groups than in the control group (Figure 2B, D, G, H). The reason can possibly be explained by the findings of Bearden and Cooke.[37] In their study, which quantified the effects of increased concentrations (approximately 7.5–30 mg/mL) of Ni (as $NiCl_2 \cdot 6\ H_2O$) on the growth and morphology of cultured 3T6 embryo mouse fibroblasts, they demonstrated that lower concentrations of Ni may produce slight stimulation of cell growth. However, a depression of the cell growth rate with morphological changes was noted at high concentrations (approximately 15–30 mg/mL) of Ni.[37]

It was reported that NiTi superelastic alloy exhibits good corrosion resistance in saliva and saline solutions.[38] The Ti alloy forms Ti oxide, which resists corrosion. Certain NiTi arch wires are manufactured using an ion implantation technique. Nitrogen ions are introduced into the near-surface region of the arch wires in an attempt to reduce the amount of friction occurring between brackets and arch wires. The coating probably also increases the corrosion resistance of the wire.[39–41] The present study revealed that U2OS cells exposed to extracts of NiTi wire in a 0.2% NaF, pH 3.5 solution showed different survival rates ($P < .05$) (Figure 3C, F). The survival rate of the 0.2% NaF, pH 3.5 group was lower than that of the control group. Jia et al[39] showed that the concentration of Ni released by orthodontic arch wire was 700 times lower than the concentration necessary to elicit a cytotoxic reaction in human peripheral blood mononuclear cells. Their results are contrary to those of the present study.

The present results showed that U2OS cell were cytotoxic to corrosion solutions, but in vitro methods do not simulate in vivo conditions. Harzer et al[42] confirmed the cytotoxicity of the in vitro studies, but in vivo study has shown that the effects are minor. Clinically, the side effects of an orthodontic treatment patient using fluoride-containing materials should be further investigated by in vivo study.

CONCLUSIONS

- The corrosive solution of the extracts of either SS or NiTi wires in acidified NaF artificial saliva can cause U2OS cell toxicity.
- Care must be exercised when fluoride-containing prophylactic agents are used on orthodontic patients. The best way to prevent toxicity is to change the wires after fluoridation or to remove the wires when applying fluoride.

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