Ion release and cytotoxicity of stainless steel wires

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SUMMARY Heat treatment is generally applied to orthodontic stainless steel (SS) wires to relieve the stresses that result from their manipulation by orthodontists. The quality and thickness of the oxide films formed on the surface of heat-treated wires can vary, and it is believed that these oxide films can influence the properties of heat-treated wires. The aim of this study was to investigate the influence of heat treatment and cooling methods on the amount of metal ions released and to examine the cytotoxicity of heat-treated wires.

In this study, four types of SS wires (Remanium, Permachrome, Colboloy and Orthos) with a crosssectional area of 0.41 × 0.56 mm were investigated. These wires were heat-treated in a vacuum, air, or argon environment, and were cooled in either a furnace or a water bath. Four control groups and 24 experimental groups were classified according to the type of wires, heat treatment conditions and cooling methods. In each group, the amount of nickel released as well as its cytotoxicity was investigated. The concentration of dissolved nickel ions in artificial saliva was measured for a period of up to 12 weeks.

In all groups, the concentration of dissolved nickel ions in artificial saliva was lowest for the vacuum heat treatment-furnace cooling group and a significant difference was shown compared with the other experimental groups. The concentration of dissolved nickel ions in artificial saliva was highest in the groups heat-treated in air (P < 0.05), while the amount of nickel released was highest in the Remanium and Colboloy (P < 0.05). The cytotoxicity was mild in all the experimental groups but the response index of the air groups was slightly higher than in the other groups.

According to these results, SS wires retain their high corrosion resistance and low ion release rate when heat-treated in a vacuum and cooled in a furnace.

Introduction

When orthodontic stainless steel (SS) wires are manipulated, their mechanical properties can change as a result of internal stress in their attempt to return to their original forms. Therefore, stress relief heat treatment, which enhances the elasticity and stability of the wire in terms of the form and the number of dislocations, must be applied.

There have been numerous studies on orthodontic SS wires. Funk (1951) reported that stress relief for orthodontic SS wire is quite effective, and suggested 450°C to be the optimum temperature for stress relief treatment. Ingerslev (1966) found that heating 18-8 SS wire at 350 to 375°C for 20 to 25 minutes was most effective, and Marcotte (1973) showed that the properties of springs made of SS improved after they were heated for 11 minutes at 400°C. In addition, Backofen and Gales (1952) reported the effectiveness of stress relief treatment, and Howe et al. (1968) studied the relationship between stress relief heat treatment and the mechanical properties of a SS wire. Lane and Nikolai (1980) reported that heat treatment could affect the mechanical properties of SS wires, while Thurow (1982) showed that heating a SS wire decreases the danger of breakage due to residual stress and stabilizes the form of the steel wire by enhancing its elasticity. Nikolai (1985) reported that steel wires that have been deformed plastically by cold working experience greater stress relief and increased ductility, but

that the effects can differ according to the type of wire, the diameter, and the degree of work hardening.

Some reports have shown that stress relief heat treatment for SS wires causes oxidation on the surface and creates an uneven oxide film, which eventually leads to localized corrosion (Hwang et al., 2001; Oh et al., 2002). Corrosion resistance is important when choosing a metallic orthodontic appliance. This is one reason why there are a limited number of materials used for making orthodontic appliances. Edie et al. (1981) studied the corrosion of nitinol and SS wires, and Lee et al. (1985) and Hero et al. (1987) the corrosion of nickel-based orthodontic wires. Toms (1988) reported that corrosion of orthodontic appliances could have serious clinical implications, ranging from a loss of dimension resulting in lower forces being applied to the teeth, to stress corrosion failure of the appliance. In addition, the possible production of toxic corrosion products in the surrounding tissues is undesirable. Lugowski et al. (1991) suggested that among the metal elements, nickel is toxic to animals and humans as well as being a carcinogen of the respiratory system and nasal cavity. The ions released from SS are mostly nickel and chrome. Nickel acts as a strong immunological reaction medium (Ricketts, 1979; Arvidson and Johansson, 1977) and may cause hypersensitivity reactions, contact dermatitis, asthma, and moderate cytotoxicity (Block and Yeung, 1982; Fisher et al., 1982; Bencko, 1983; Bass et al., 1993). Fernandez *et al.* (1986), Spiechowicz *et al.* (1984) and Romaguera *et al.* (1988) reported that prostheses made of a nickel alloy cause a burning sensation in the oesophagus and neck area and a loss of taste. Van Loon *et al.* (1984) found that it caused contact stomatitis. In orthodontics, Greig (1983) and Dickson (1983) reported the development of contact dermatitis from headgears, and Rickles (1980) and Levy *et al.* (1980) the occurrence of nickel allergies from orthodontic appliances. In addition, Haudrechy *et al.* (1994; 1997) found that dermatitis can occur in patients sensitive to the nickel ions released from SS.

It is believed that the oxide film created from the heat treatment of orthodontic wires, which is undertaken in order to increase the strength and hardness as well as to relieve internal stress, will affect the corrosion resistance of the wire and the amount of metal ions released. Clinically, heat treatment of orthodontic wires is normally carried out in air at a high temperature for a short period of time. The cooling methods of the steels vary widely.

The aims of this study were, therefore, to compare and analyse the effects of each heat treatment environment and cooling method on the concentration of dissolved nickel ions in artificial saliva as well as the cytotoxicity of the wires.

Materials and Methods

Four types of orthodontic SS wires were included in this study: Remanium (Dentaurum, Ispringen, Germany), Permachrome (3M Unitek, Monrovia, USA), Colboloy (G&H, Greenwood, USA), and Orthos (Ormco, Glendora, USA). All the products were supplied as straight lengths with cross sectional areas of 0.41×0.56 mm.

Heat treatment was performed in three different environments: vacuum, air, and argon. Two different cooling methods were also chosen: furnace- and water-cooling. The temperature for heat treatment was maintained at 500°C for 6 minutes; the wires were placed in a 20°C furnace and the temperature was increased at a rate of 10°C/minute. The vacuum was set to 1×10^{-5} Torr for the heat treatment in a vacuum. For argon treatment, a continuous flow of argon gas was used and the wires were kept in the furnace at 800 Torr. For water-cooling rate the wires were quenched in a water bath at 5°C/minute.

For each experimental group, 30 specimens with a nominal length of 5 cm were cleaned ultrasonically with ethyl alcohol and acetone, rinsed with distilled water, and then dried. In each dish, six specimens were immersed in 50 ml of artificial saliva (Table 1), sealed, and then stored in a 37°C incubator containing 5 per cent CO_2 . Five millilitres of artificial saliva were extracted from the bottle on day 1 and 3, and weeks 1, 2, 4, 8 and 12. The concentration of nickel ions in the saliva was measured using a Graphite Furnace Atomic Absorption Spectroscope (Model 6601, Shimadzu Co. Ltd., Kyoto, Japan).

 Table 1
 Composition of artificial saliva.

Constituent	Concentration (g/l)		
NaCl	0.40		
KCl	0.40		
CaCl ₂ ·2H ₂ O	0.795		
NaH ₂ PO ₄ ·2H ₂ O	0.780		
Na ₂ S·9H ₂ O	0.005		
$CO(NH_2)_2$ (Urea)	1.0		
Distilled water	1000 ml		

After the wires had been immersed in artificial saliva for 12 weeks, they were removed and dried, and then observed with a Hiscope (Hirox/KH 1000 model, Micro Hiscope System, Tokyo, Japan) to determine the changes and extent of corrosion on the surface.

For cytotoxicity testing, four specimens were prepared for each experimental group and were processed so that a surface area of 1 cm² came into contact with the agar. They were sterilized using ethylene oxide gas, and cleaned with distilled water for sterilization. Copper alloy (NPG, Aalbadent Co., Cordelia, California, USA) was used as a positive control, and polyethylene as a negative control. The supernatant solution was produced by cultivating L-929 cells, a mouse fibroblast cell-line, in α-MEM medium. A supernatant solution (10 ml) in the α -MEM medium was added to a Petri dish and cultivated for 24 hours. The α -MEM medium was then removed, and 10 ml of Eagle's agar medium at 45 to 50°C was added to each Petri dish, which were left to stand at room temperature for 30 minutes. After the Eagle's agar medium had solidified, a neutral red vital stain solution (10 ml) was added slowly to the centre of the dish. This was then spread over the surface, and the sample was left to stand for 30 minutes. Immediately after removing the dying solution, the specimens were placed in contact with the agar and incubated for 24 hours in a 37°C, 5 per cent CO₂ incubator. First, a Petri dish was placed on top of a white paper, and then the zone index was measured after observing the size of the discoloured area. The lysis index was determined by calculating the lysed ratio of the cells in the discoloured area with an inverted phase contrast microscope (CK2, Olympus, Tokyo, Japan). Finally, the response index [ISO 7405:1997(E)] was measured by averaging the zone and lysis indices of the four specimens:

Response index = Zone index / Lysis index

The concentration of dissolved nickel ions in the artificial saliva was compared based on each group's type, heat treatment method, and immersion time in the artificial saliva. For the groups that had the same immersion time, the Kruskal–Wallis test was used to compare the concentration of nickel ions released for the different cooling methods, and the Tukey grouping method to compare the concentration of nickel released for the

different heat treatment conditions and types of wires. Statistical significance was set at 95 per cent.

Results

The concentration of dissolved nickel ions in the artificial saliva from the heat-treated wires that had been immersed in artificial saliva for 12 weeks are shown in Figure 1.

The samples heat-treated in a vacuum showed significant differences for each cooling method. The furnace-cooled group showed a much lower nickel ion concentration than the water-cooled group, and all four wires showed a statistically significant difference for each immersion time. In the furnace-cooled group, the concentration of dissolved nickel ions in the artificial saliva from the Remanium sample ranged from 31.90 ng/ml (day 1) to 55.53ng/ml (week 12), and the concentration of the other samples ranged from 1.84 ng/ml (day 1) to 16.28 ng/ml (week 12). On the other hand, the concentration of dissolved nickel ions in the artificial saliva from the water-cooled group ranged from 47.49 ng/ml (day 1) to 156.54 ng/ml (week 12). In contrast to the vacuum group, the air group showed a less significant difference in the concentration of nickel ions for each cooling method. However, the furnace-cooling method resulted in the release of a larger amount of nickel ions than that released from the samples using the water-cooling method, and there were some immersion times where the concentration of nickel ions showed statistically significant differences. In particular, with Orthos, there was a significant difference among the immersion times. The wires that had been heated in argon showed different nickel ion concentrations depending on the wire type and cooling method. There was a larger concentration of dissolved nickel ions in the artificial saliva from the Remanium and Orthos samples when they were cooled in a furnace. In particular, Orthos showed a significant difference at all immersion times except at 12 weeks. In contrast, Permachrome and Colboloy released more nickel ions when cooled in water than when cooled in a furnace, and Colboloy showed a significant difference at all immersion times.

The wires cooled in a furnace showed a significant difference in the concentration of nickel ions released for the different heat treatment environments. All four wires showed the largest concentration of dissolved nickel ions in the artificial saliva (55.28 to 273.03 ng/ml) when heated in air, the second highest concentration of nickel ions (18.94 to 83.98 ng/ml) when heated in argon, and the least (1.84 to 55.53 ng/ml) when in a vacuum.

For the wires cooled in water, the group heated in air released the most nickel (33.01 to 212.71 ng/ml), and the group heated in argon released the least (11.13 to 119.29 ng/ml). This trend was most obvious with Orthos.

Depending on the cooling method, the wires that had been heated in a vacuum showed a significant difference. When cooled in a furnace, Remanium released more nickel and showed a greater significant difference than the other three types of wires. In contrast, Colboloy released more nickel when cooled in water.

When the wires were heated in air, Colboloy released the most nickel regardless of the cooling method, closely followed by Remanium.

When heated in argon and cooled in a furnace, Remanium released the largest amount of nickel ions, Colboloy and Permachrome an intermediate concentration, and Orthos the least. Among the water-cooled group, Colboloy released the largest amount of nickel, followed by Remanium, Permachrome, and Orthos.

Observation of the wires after 12 weeks of immersion in artificial saliva with a Hiscope demonstrated that in the control group and the vacuum heat treatment-furnace cooling group the surfaces were relatively smooth and clean, while the other groups showed obvious corrosion. In particular, the surface in the air heat treatment group was very uneven and tainted (Figure 2).

An agar overlay test was performed in order to determine the toxicity of the heat-treated steels on the cellular scale. Both the control and experimental groups showed rather low toxicity, and the response index ranged from 1/1 to 1/3. Overall, there was low toxicity but the air heat treatment group showed a slightly higher reaction than the others (Table 2).

Discussion

The mechanical properties of materials improve when they are cold worked, but internal stress forces the material to return to its original form. Clinically, when an orthodontist applies manipulation such as a loop, helix, or arch forms, the materials' internal stress reaches a very high level, and requires heat treatment. In terms of metallurgy, this stress relief heat treatment is called 'recovery'. It is a process that does not cause grain growth and removes only the minor stress existing within the grains. Stress relief heat treatment is best applied at low temperatures for a long time (Gjerdet and Hero, 1987). According to the literature, as the temperature for stress relief heat treatment is increased, the wire surface creates a more unstable oxide film, causing more corrosion and surface roughness (Gjerdet and Hero, 1987). However, stress relief heat treatment is mostly carried out at high temperatures over a short period of time, and the cooling methods also vary.

SS and chrome-containing alloys such as cobalt-chrome and nickel-chrome, do not corrode easily because of the electrochemically formed passive film. Chrome affects the resistance to corrosion, which increases as the chrome content increases. The mechanism occurs by creating a thin, elaborate oxide film, which delays corrosion. This passive film offers protection against aggressive ions in the air and prevents corrosion. When forming chrome oxide on a metal surface to protect it from discolouration and corrosion,



Figure 1 Amount of nickel ion released from (a, b) Remanium, (c, d) Permachrome, (e, f) Colboloy, and (g, h) Orthos according to immersion time in artificial saliva. * indicates Kruskal–Wallis test: significant difference between cooling methods in same period. a, b, c indicate Tukey grouping method: significant difference between heat treatment conditions in the same period (P < 0.05). FC, furnace-cooling; WC, water-cooling.

		Remanium	Permachrome	Colboloy	Orthos
Control					
Furnace	Vacuum-		9		
Water	Vacuum-				
Furnace	Air-				
Water	Air-				
Furnace	Argon-				
Water	Argon-				

Figure 2 Surface morphology of the wires after ion release testing for 12-weeks immersion. Furnace, furnace-cooling; Water, water-cooling.

Types	Heat treatment condition	Cooling method	Zone index	Lysis index	Response index
Remanium		Control	0~1	0~1	0/1
	Vacuum	Furnace	1~1	1~1	1/1
		Water	1~1	1~1	1/1
	Air	Furnace	1~1	0~1	1/1
		Water	1~1	1~2	1/2
	Argon	Furnace	1~1	1~1	1/1
	5	Water	1~1	1~1	1/1
Permachrome		Control	0~1	0~1	0/1
	Vacuum	Furnace	1~1	1~1	1/1
		Water	1~1	1~1	1/1
	Air	Furnace	1~1	1~2	1/2
		Water	1~1	1~1	1/1
	Argon	Furnace	1~1	1~1	1/1
	0	Water	1~1	1~1	1/1
Colbolov		Control	0~1	0~1	0/1
5	Vacuum	Furnace	1~1	1~1	1/1
		Water	1~1	1~1	1/1
	Air	Furnace	1~1	1~1	1/1
		Water	1~1	2~3	1/3
	Argon	Furnace	1~1	1~1	1/1
	0	Water	1~1	1~1	1/1
Othos		Control	0~1	0~1	0/1
	Vacuum	Furnace	0~1	0~1	0/1
		Water	1~1	1~1	1/1
	Air	Furnace	1~1	2~3	1/3
		Water	1~1	1~2	1/2
	Argon	Furnace	1~1	1~1	1/1
	5	Water	1~1	1~1	1/1
Positive control (copper alloy)			3~4	3~4	3/4
Negative control (polyethylene)			0	0	0/0

 Table 2
 Cytotoxicity of the heat-treated stainless steel wires.

the chrome oxide must be maintained and kept stable throughout the entire material. However, when SS is heattreated, surface oxidation can occur, and an uneven oxide film may cause localized corrosion. Each heat treatment environment and cooling method can affect the thickness and form of the uneven oxide film on the wire surface, which can create various degrees of corrosion. Therefore, this study examined the changes in the property and nickel ion release for different heat treatment environments and cooling methods.

A comparison was made across three different heat treatment environments to evaluate the results based on the different cooling methods. The vacuum heat treatmentfurnace cooling group released less nickel than the vacuum heat treatment-water cooling group, and all four types of wires showed a significant difference for each immersion time. The reason the vacuum heat treatmentfurnace cooling group released a small amount of nickel was due to the oxide film formed on the wire surface. It is believed that because a thin, even oxide film had formed during the heating and cooling process, the high corrosion resistance was maintained. The reason the vacuum heat treatment-water cooling group released a large amount of nickel is believed to be because during the cooling process, after being heated in vacuum, the wire surface comes in to contact with water molecules, which react violently, creating an unstable oxide film.

For the air heat treatment group, the effects of each cooling method were different from the vacuum heat treatment group. In most cases, the concentration of dissolved nickel ions in the artificial saliva was greater in the furnace-cooling group than in the water-cooling group and some significant difference was shown. It is believed that being heated in air and cooled in water for a long time results in more stability than being continuously exposed to air molecules. It is also considered to be because Remanium is a type 302 SS. It contains the most carbon of the four types of wires, which serves as an unstable condition to an oxide film created during heat treatment. Colboloy is a type 304 SS, like Permachrome and Orthos, but there could be variations in the evenness and stability of the surface oxide film due to different production procedures. When heated in argon, an inert gas, each type of wire showed different results. Remanium and Orthos released more nickel when cooled in a furnace, and Permachrome and Colboloy released more when cooled in water.

Corrosion is an electrochemical process, which changes the basic properties of a metal and occurs due to the loss of metal ions or the gradual dissolution of the oxide or sulphide film. Corrosion accelerates as a result of the damage to the passive film, but because of the effects of the different production processes, heat treatment, metal abrasion, and the environment, even metals with the same composition can show a different degree of corrosion. Within the oral cavity, many factors work together to create an environment that makes aqueous corrosion in metals and alloys more favourable. Saliva acts as an electrolyte, which can cause corrosion, and also as a medium for chemical reactions between metals. The organic acids and enzymes that microbes produce or the bacteria existing within the mouth can also cause corrosion. In addition, factors such as low acidity, sudden temperature changes, dental plaque, and proteins can also cause corrosion.

Because the corrosion products from orthodontic appliances can be harmful as a result of absorption by the surrounding structure or body, it is important to measure the actual amount of the metal released after the appliance is placed in the mouth. The factors that can affect the amount of metal released from orthodontic appliances include the corrosion resistance of the material, the brazing or welding effects on the metal, galvanic corrosion of dissimilar metals, the interaction between microbacteria within the dental plaque, the surface of the appliance, and how much the appliance's surface is finished. (Maijer and Smith, 1982; 1986). There have been many measurements of the amount of metal released from orthodontic appliances under various physical and chemical conditions (Berge and Gjerdet, 1982; Bishara et al., 1993; Barrett et al., 1993; Kerosuo et al., 1995). The SS currently used in orthodontic clinics is of type 302 or 304, both of which contain 8 to 10 per cent nickel. Nickel is added to maintain the steel's face centred cubic structure, and is created when heated at 912°C or higher. Nickel also increases the strength, ductility, and resistance to general, crevice and erosion corrosion. However, there are problems associated with patients using metal appliances such as allergic reactions to nickel. In addition, there are reports (Dickson, 1983; Greig, 1983; Levy et al., 1980; Rickles, 1980) showing that there can also be hypersensitive reactions to orthodontic appliances made of SS.

It is known that hypersensitivity to nickel can often occur in the contact area and is relieved when the cause is removed. It occurs frequently in females, and other body parts can also show hypersensitivity when nickel is absorbed by saliva or the soft/hard tissues (Bergman *et al.*, 1980; Magnusson *et al.*, 1982). In addition, it has been reported that orthodontic treatment-related severe gingivitis can be an effect of poor oral hygiene and the release of nickel and chrome from SS (Schriver, 1976).

The amount of nickel measured in the experiments was quite large, except for the control and vacuum heat treatment groups, particularly when compared with a human's daily average consumption of food (5 to $10 \mu g/l$)

and beverages (0.43 μ g/l). However, within the oral cavity, many factors may increase this amount. Because orthodontic appliances are placed for 2 to 3 years and symptoms can appear years later, it is better to minimize the amount of nickel ion released. In addition, there is a need for more long-term investigations because there is still some controversy as to whether or not there is hypersensitivity towards nickel-containing wires. There are also questions regarding issues such as hypersensitive patients' resistance to orthodontic treatment and relapse, and side-effects due to cytotoxicity reactions (Dunlap *et al.*, 1989; Van Hoogstraten *et al.*, 1991).

Conclusions

This study investigated the amount of nickel released as a result of different heating and cooling methods used in orthodontic clinics when applied to four different types of wires (Remanium, Permachrome, Colboloy, and Orthos). The following results were obtained:

- 1. The vacuum heat treatment-furnace cooling group showed a similar concentration of dissolved nickel ions after being immersed in artificial saliva, while the air heat treatment group showed a significant difference (P < 0.05) regardless of the cooling method used.
- 2. The concentration of dissolved nickel ions in the artificial saliva from the Remanium and Colboloy wires showed, in most cases, the greatest difference regardless of the conditions.
- 3. The result of the cytotoxicity experiment showed mild toxicity in all groups but the air heat treatment group showed a slightly higher level.
- 4. SS released the lowest amount of nickel when heattreated in a vacuum and slowly cooled in a furnace.

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