# The effect of electro-thermal treatment of stainless steel arch wire on mechanical properties and cell proliferation

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*Background:* Electric resistance heat treatment may be performed on orthodontic arch wires, but it remains unclear whether this procedure reliably provides better mechanical properties or whether an austenite phase transition affords less cytotoxicity and less arch wire width change.

*Methods:* Stainless steel (SS) arch wires of  $0.017 \times 0.025$  and  $0.019 \times 0.025$  inches in size were heat-treated using a spot welder for eight seconds at power settings of 2, 4, 6 and 8. The surface morphology, coefficient of friction (COF), flexural modulus, cytotoxicity, austenitic content, colour change and arch width of the samples were subsequently analysed. *Results:* The COF, flexural modulus and austenitic content of the orthodontic SS arch wires increased after heat treatment. SS wires appearing brownish-yellow and blue exhibited higher flexural moduli. The heat treatment of the SS wires did not significantly increase arch wire width or cause cytotoxicity.

*Conclusions:* Electric resistance heat treatment of SS arch wires is a feasible method to improve the flexural modulus without widening the arch wire and increasing cytotoxicity. The colour of the wire helps determine the heating status, and the maximum flexural modulus of the wires is obtained when the colour changes to brownish-yellow. (Aust Orthod | 2020; 36: 75-86)

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## Introduction

Arch wires are an integral part of fixed appliance orthodontic treatment and treatment results are highly correlated with the structural and mechanical properties of the component metals.<sup>1</sup> Stainless steel (SS) is a serviceable wire alloy due to its excellent combination of mechanical properties, corrosion resistance and cost.<sup>2</sup> Orthodontic SS arch wires are usually American Iron and Steel Institute (AISI) types 302 and 304 austenitic SS.<sup>3-5</sup> The manufacture of SS wires under a cold working process, involving rolling, drawing and pressing, may cause crystal dislocations, point defects and metallic phases. The resulting cold work may therefore alter the elasticity and mechanical properties of the wire, resulting in increased brittleness.<sup>6</sup> As a consequence, after the drawing process and the formation of loops, springs, and archforms, the wire may be heat-treated at a lower temperature to relieve some of the stress.<sup>5</sup> The heat treatment improves the elasticity, dislocation stability and arch shape.<sup>5,7,8</sup> Conventionally, however, wires have been heat-treated in air at higher temperatures for a few minutes.

The use of electrical heat treatment for orthodontic arch wires is advocated<sup>9-13</sup> and dental spot welders are commercially available to deliver the electrothermal treatment. In recent years, research assessing electrothermal treatment has gradually increased<sup>9-13</sup> and identified problems that currently remain unresolved. A uniform treatment heating time/ power setting standard that corresponds to a heating temperature is unavailable. The outcome of the heat treatment is usually determined based on clinical experience, governed by the surface colour of the arch wire.<sup>14,15</sup> This renders the properties of the heattreated arch wire largely unknown. Secondly, the performance of the arch wire after heat treatment is related to the heating and cooling conditions. An electrothermally treated arch wire is heated and air cooled, which creates a rapid cooling rate compared with furnace cooling.<sup>16,17</sup> No studies have confirmed whether the heating and cooling method exerts a negative effect on the structural and mechanical properties of orthodontic SS. Thirdly, as a working arch wire for space closure, an SS arch wire is placed in the mouth and in use for a long time. Clinicians have not determined whether a heat-treated SS wire exerts a cytotoxic effect on gingival fibroblasts. Finally, the shape of the SS arch wire determines dental arch form, and this is a critical factor in affecting stability after active orthodontic treatment.<sup>18</sup> No research has confirmed whether the width of SS arch wires is significantly altered by electrothermal treatment or whether the arch wire shape must be adjusted after heat treatment.

Since the electric resistance heat treatment procedure is a desirable pre-insertion procedure, the main aims of the present study are: (1) to study the effects of different electrothermal treatment conditions on the bending properties and phase transition of SS wires and the colours of the arch wires under different electrothermal treatment conditions, while exploring the feasibility of using colour as an indication of the degree of the electrical heat treatment; (2) to study the cytotoxic effect of electrothermally treated SS arch wires on gingival fibroblasts and determine the biological safety following heat treatment; and (3) to analyse the change in arch wire width after heat treatment and determine the necessity of adjusting the subsequent shape of the arch wire.

## Materials and methods

### Materials

Commercially prepared orthodontic 304 SS wires of sizes of  $0.017 \times 0.025$  inches and  $0.019 \times 0.025$  inches (TP Orthodontics, Inc., IN, USA) were used in the present study. All were held using pliers at both ends and heat-treated for eight seconds using a spot welder (SMACO, SDH-2000, Zhejiang, China) at power

settings of 2, 4, 6 and 8 (the welding machine has nine power settings, with number 1 referring to the lowest and 9 referring to the highest voltage setting; Power supply: AC 220 V, 50 Hz; Output power: 2000 W; Output voltage: 5 V). Unheated arch wires were used as control samples. Six samples of both  $0.017 \times 0.025$ and  $0.019 \times 0.025$  inch arch wires were included in each heat treatment group.

# Surface analysis and coefficient of friction (COF) measurement

The morphology of the surface of the SS wire before and after heat treatment was examined using scanning electron microscopy (SEM) at 1000× magnification.<sup>19</sup> The COF of the SS arch wires was tested using a Universal Micro Tribometer (UMT-2, Universal Micro Tribometer, Center Tribology, CA, USA). Nine-centimetre SS wire segments, as test samples, were attached to UMT-2 plates using dental resin (3M, USA). A 304SS plate was created and sufficiently polished to represent the grooved surface of an SS bracket and was connected to an electric motor. The electric motor pulled the SS plate through the SS arch wire at a speed of 6 mm/min for one minute using a vertical force of 1.47 N. The experiment was performed in simulated saliva at a temperature of 25°C. The COF was calculated by averaging the COFs recorded between the 10th and 60th seconds, including 10,000 test data points. Six arch wires were included in each group.

The surface roughness of an unheated SS wire was also tested under the same conditions.

## Cells and cell culture

Primary human gingival fibroblasts were purchased from the Sciencell Research Laboratory (Cat No. 2620, Carlsbad, CA, USA) and cultured in fibroblast culture medium consisting of basal medium, 2% foetal bovine serum (FBS), 1% fibroblast growth supplement (FGS) and 1% penicillin/streptomycin (P/S), followed by incubation in an atmosphere of 5% CO<sub>2</sub> and 95% air at 37°C. Cultured cells from passages 3–4 exhibiting a spindle shape were considered suitable for the subsequent experiment.

The method used to prepare the sample extract adhered to the guidelines for the biological evaluation of national standard medical devices of the People's Republic of China (GB/T 16886.12-2005/ International Organization for Standardization (ISO) 10993-12:2002). The unheated and heat-treated arch wires were sterilised with ultraviolet irradiation, and a leaching solution was prepared using fibroblast culture medium with a concentration of 0.1 g/ml. After 24 hours of culture at 37°C in a 5% CO<sub>2</sub> incubator, the extract was prepared by sterilisation through a microporous membrane filter.

## Cytotoxicity

Cytotoxicity induced by orthodontic appliances is related to metal ion release from corrosion processes and has become an important issue in the assessment of orthodontic biosafety.<sup>20</sup> Corrosion products leach out of orthodontic appliances in sufficient amounts to exert local cytotoxic effects.<sup>21,22</sup> The ISO (International Organization for Standardization) lists guidelines for the biological evaluation of medical devices and biomaterials (ISO 10993–5). The principle of this method is to immerse the bioactive material in a suitable medium to release any active elements. Cells are then cultured with the active elements and the growth of the cells is monitored to evaluate the biocompatibility and biological behaviour of the material.<sup>20</sup>

Gingival fibroblasts were inoculated (40,000 cells/ well) in 24-well plates and cultured with a prepared, material-leaching, fibroblast culture medium sample. After one, three, five and seven days of incubation, the medium was replaced with 100 (3-(4,5-Dimethylthiazol-2-yl)-2,5ul/well MTT diphenyltetrazolium bromide) solution (5 mg/ml) and 900 µl/well culture medium. Seven hundred and fifty microliters of dimethyl sulfoxide were added to each well to dissolve the coloured formazan product. Each experiment was performed in triplicate. The results were reported in units of optical density (OD) and the absorbance was measured at 490 nm using an

Table I. Evaluation of cytotoxicity.

enzyme-linked immunosorbent assay (ELISA) reader (uQuant; Bio-Tek Instruments, Inc., CA, USA). The relative growth rate (RGR) was calculated using the following formula: RGR (percent) = (OD treatment/ OD control) × 100. The cytotoxicity of the samples was evaluated according to the criteria<sup>23</sup> from The United States Pharmacopeia, as shown in Table I.

### **Bending** properties

A three-point bending test was performed on the sample wires (N = 6). All samples were loaded using the same protocol, and testing was performed in accordance with ISO/CD 15841.24 A universal testing machine (Shimadzu, Japan) was used to measure the flexural modulus using a wire span of 20 mm and at a crosshead speed of 1 mm/min. The tangent modulus of elasticity (commonly referred to as the "modulus of elasticity") is defined as the ratio of the stress within the elastic limit to the corresponding strain. This ratio is calculated by plotting the tangent to the steepest initial straight line portion of the load-deflection curve and applying the equation:<sup>25</sup> EB =  $L^3m/4bd^3$ , where EB represents the modulus of elasticity in bending (MPa), L represents the support span (mm), b represents the width of the tested beam (mm), d represents the depth of the tested beam (mm) and m represents the slope of the tangent to the initial straight-line portion of the load-deflection curve (N/mm).

## X-ray diffraction (XRD)

XRD was used to identify the phase of the wire samples. An electric XRD diffractometer (Bruker AXS D8, Bruker, Germany) was used to analyse the XRD patterns of the unheated and heat-treated wires. For each experiment, wire was cut into 0.4 inch pieces, which were then placed side by side on tape to form a plate sample with an overall size of  $0.4 \times 0.4$  inches. Copper-Ks radiation (h = 1.54 A) and a graphite monochromator were used with an excitation voltage

Cytotoxicity grade	Relative growth rate (%)	Cytotoxicity
0	≥100	No
]	80-99	No
2	50-79	Slightly
3	30-49	Midrange
4	0-29	Obvious

of 40 kV and a current of 12 mA. A fast scan rate of 20 at 2°/min was applied. A series of diffraction peak indices were assigned to the face-centred cubic crystal structures or body-centred cubic phases present in these SS wires using standard procedures. A semi-quantitative analysis<sup>26</sup> of the residual austenitic content in the SS wires was performed using TUX software, with the austenite crystal peaks at (200), (220), and (311) and the martensitic crystal peaks at (200) and (211) serving as reference points to analyse the phase content.

#### Colour measurement

Previous studies have shown the potential of using digital cameras for colour matching in dentistry.<sup>27,28</sup> The combination of a commercial single-lens reflex (SLR) digital camera with an appropriate calibration scheme has proven to be reliable and advantageous for colour reproduction.<sup>29</sup> A high-resolution digital SLR camera (Nikon D1, Japan) equipped with a chargecoupled device was attached to a table. The camera was fitted with a 105 mm macro lens (Nikon, Japan) with a magnification of 1:1, and the lens was oriented perpendicular to the measuring surface of the sample. A D-65 table lamp (Panasonic, Japan) was mounted on an optical table to view and provide standard illumination of the object. The capture distance was set to 13 cm, which allowed the entire shadow guide to be sufficiently placed within the image. The white balance of the camera was manually preset by capturing an image of a white standard plate according to the manufacturer's instructions. The same shadow guide was used to capture images at a fixed shutter speed (1/200 seconds), f-stop (F25), and ISO (100). All other settings of the camera were set to auto/default mode. The digital images were analysed using Adobe Photoshop 6.0 software (Adobe Systems, USA), and the image mode was changed from RGB to L\*a\*b\*. Each group included six arch wires, and the L\*a\*b\* values (Lab histogram) of three randomly selected points on each arch wire were measured; the average L\*a\*b\* value of nine points from each size arch wire under each heat treatment condition was calculated. The colour difference or  $\Delta E$  is defined by the following formula:  $\triangle E = (\triangle L^{*2} + \triangle a^{*2} +$  $(\Delta b^{*2})1/2$ , where  $(\Delta L^*)$ ,  $(\Delta a^*)$  and  $(\Delta b^*)$  indicate the respective differences between the measured CIE LAB values of any two groups.

#### Transverse arch wire widths

Using a diagram depicted on A4 paper, the lateral width of the arch wire at the front and back of the wire was plotted to analyse the occurrence of lateral changes in these regions.<sup>14</sup> Initially, a horizontal line perpendicular to the vertical centre line was drawn at the end of the arch wire as a reference limit. Subsequently, a new horizontal line was drawn along the previous line and parallel to the anterior end of the arch wire, creating a reference for the front end of the wire. Two straight lines were then drawn parallel to the foremost parallel line and were located 10 mm and 30 mm from the front parallel line. A standard map dividing the four points corresponding to the intercanine width and the intermolar width was subsequently obtained, as illustrated in Figure 7(A).

Each arch wire was individually superimposed on the diagram such that the points corresponding to the intermolar and intercanine widths could be measured. The anterior and posterior regions of the arch wires were measured using electronic Vernier callipers. The arch wire size was recorded for six random samples from the same group. The intermolar and intercanine widths of each wire were measured five times at 10-minute intervals.

## Statistical analysis

All data are presented as the mean  $\pm$  standard deviations (SD). Differences in each type of wire were determined using one-way ANOVA with the Student-Newman-Keuls multiple comparison test. A confidence level of 95% (p < 0.05) was considered statistically significant. The statistical analysis was performed using SPSS v.17.0 software (SPSS).

### Results

## Surface morphology

As shown in Figure 1, no significant differences were observed in the SEM images of the various arch wires after heat treatment over the different times. The surface of the arch wire showed a longitudinal texture because some local defects were created during the manufacturing process. No obvious pit defects were observed on the surface of the arch wires after heat treatment. However, the number of surface impurities increased with increasing heat treatment temperatures.

#### COF

As shown in Figure 2, the surface COF test results for the  $0.017 \times 0.025$  inch SS wire showed higher COF values for the specimens that received heat treatments at power settings of 6 and 8 for eight seconds than for the unheated SS wire (p < 0.05). However, significant differences in the COF values were not observed between the SS specimens treated with heat at a power setting of 2 or 4. No significant differences in the COF were observed between the SS specimen treated with heat at a power setting of 2 or 4 and the unheated SS wire. For the  $0.019 \times 0.025$  inch SS wire, the surface COF of the SS wire subjected to heat treatment at a power setting of 8 was significantly different from untreated wires or wires treated at the other power settings (p < 0.05). The COF values of the SS wire subjected to heat treatment at power settings of 2, 4

and 6 were not significantly different from the COF of the untreated wire.

#### Cytotoxicity

As shown in Figure 3, the extracts of the 0.017 × 0.025 inch SS wire heated at power settings of 6 and 8 significantly inhibited the proliferation of gingival fibroblasts on the third, fifth, and seventh days, and the difference was statistically significant (p < 0.01). For the 0.019 × 0.025 inch SS wire, the extracts of the SS wire heated at power settings of 4, 6 and 8 significantly inhibited the proliferation of gingival fibroblasts on the third and seventh days, and the difference was statistically significant (p < 0.01). Based on the calculated RGR, the heat-treated 0.019 × 0.025 inch SS arch wire extract was slightly cytotoxic towards



Figure 1. SEM images of unheated SS wire specimens and heated specimens. (A1-A5) Unheated 0.017 × 0.025 inch SS wire specimens and specimens heated at power settings of 2, 4, 6, and 8 for eight seconds. (B1-B5) Unheated 0.019 × 0.025 inch SS wire specimens and specimens heated at power settings of 2, 4, 6, and 8 for eight seconds.



Figure 2. The COF of unheated and heated SS wire specimens. (A) Unheated  $0.017 \times 0.025$  inch SS wire specimens and specimens heated at power settings of 2, 4, 6, and 8 for eight seconds. (B) Unheated  $0.019 \times 0.025$  inch SS wire specimens and specimens heated at power settings of 2, 4, 6, and 8 for eight seconds. (C) Illustrative artwork of the experiment setup.



Figure 3. The effect of heat-treated SS arch wire extracts on the proliferation and RGR of human gingival fibroblasts. MTT assays show the effect of heat-treated SS arch wires on the proliferation of human gingival fibroblasts on days 1, 3, 5 and 7. Cells cultured with the unheated SS arch wire extract served as the control group. \*p < 0.05 and \*p < 0.01.

gingival fibroblasts on day seven of culture (RGR = 75.2724). The remaining heat treatment conditions and time points did not produce cytotoxic effects.

### Flexural modulus

As shown in Figure 4, the 0.017  $\times$  0.025 inch SS arch wires heated at a power setting of 6 achieved the maximum elastic modulus, with a significant difference compared with the other groups treated at different power settings (p < 0.05). The modulus of elasticity of the arch wire was also significantly increased after treatment at power settings of 4 and 8 compared with the untreated wire (p < 0.05). No significant increase in the elastic modulus of the arch wire was observed at a power setting of 2 compared with the untreated wire. The  $0.019 \times 0.025$  inch SS arch wire achieved a maximum modulus of elasticity at power settings of 6 and 8, and although no significant difference was observed between these two groups, significant differences were identified between these groups and the other groups. The modulus of elasticity of the arch wire was significantly increased at a power setting of 4 compared with the untreated wire. No significant increase in the elastic modulus of the arch wire was observed at a power setting of 2 compared to the untreated wire.



Figure 4. (A) Flexural moduli of the unheated 0.017  $\times$  0.025 inch SS wire specimens and specimens heated at power settings of 2, 4, 6, and 8 for eight seconds. (B) Flexural moduli of the unheated 0.019  $\times$  0.025 inch SS wire specimens and specimens heated at power settings of 2, 4, 6, and 8 for eight seconds. Bars with dissimilar letters indicate significantly different values (p < 0.05). Each value is presented as the mean  $\pm$  SD (N = 6).

### XRD results

As shown in Figure 5, the XRD diffraction patterns obtained with different heat treatment times for the 0.017  $\times$  0.025 inch SS and 0.019  $\times$  0.025 inch SS wires showed austenitic crystal peaks at (111), (200), (220), (222) and (311) and martensitic crystal peaks at (110), (200), and (211) in the unheated SS wires. Thus, both the austenitic and martensitic phases are present in unheated SS wires. According to the semiquantitative analysis, the austenitic content of the 0.017  $\times$  0.025 inch SS wire increased with increasing heat treatment power settings. For the 0.019  $\times$  0.025 inch SS wire, the austenitic content initially decreased at a power setting of 2 and then increased.

### Colour shade

As shown in Figure 6,  $0.017 \times 0.025$  inch SS and  $0.019 \times 0.025$  inch SS wires were heated at different power settings for the same periods. The colour of the  $0.017 \times 0.025$  inch SS wire changed from silver to light yellow and turned brownish-yellow and finally blue. The  $0.019 \times 0.025$  inch SS wire changed from silver to light yellow, turned brownish-yellow, blue, and finally dark grey. The L\*a\*b\* colour difference values calculated between any two groups for the same size arch wire were greater than 1.

#### Arch width

As shown in Figure 7, for the  $0.017 \times 0.025$  inch SS wire, the width between the molars was significantly widened at power settings of 2 and 4 compared with the values of the other groups (p < 0.05). No

significant differences were observed in the intermolar width for the wires treated at a power setting of 6 or 8 compared with the control group. At a power setting of 2, the width between the canines was significantly increased compared with the control group (p < 0.05). At power settings of 4, 6, and 8, the width between the canines was not significantly different from the control group. For the  $0.019 \times 0.025$  inch SS wire, the width between the molars was significantly increased at a power setting of 2 compared with the control group (p < 0.05). Wires treated at power settings of 4, 6, and 8 showed no significant differences in the width between the molars compared with the wires in the control group. Wires treated at power settings of 2 and 4 showed significantly different intercanine widths compared with wires from the control group (p < 0.05), while wires treated at power settings of 6 and 8 did not show any differences.

#### Discussion

## Flexural modulus, COF and sliding resistance

Orthodontic sliding mechanics are acceptable and desirable due to their simplicity. However, the efficiency of space closure may be affected by friction and binding. Clinically, many factors cause sliding interference, including wire size, wire composition, bracket slot width, bracket composition, wire-to-slot ligation, interbracket distance, and relative interfacial motion between the arch wire and the bracket.<sup>30</sup> Thinner arch wires delay entry to the binding phase by increasing the first- and second-order critical contact angles ( $\theta$ c) compared with thicker arch wires of the



Figure 5. (A) XRD spectra of the unheated  $0.017 \times 0.025$  inch SS wire specimens and specimens heated at power settings of 2, 4, 6, and 8 for eight seconds. (B) XRD spectra of the unheated  $0.019 \times 0.025$  inch SS wire specimens and specimens heated at power settings of 2, 4, 6, and 8 for eight seconds. (C) Changes in  $\alpha$ 'austenite percentages of the  $0.017 \times 0.025$  inch SS specimens and  $0.019 \times 0.025$  inch SS specimens heated at different power settings.



Figure 6. (A) Heat-induced changes in the colour of the  $0.017 \times 0.025$  inch and  $0.019 \times 0.025$  inch unheated SS wire specimens and specimens heated at power settings of 2, 4, 6, and 8 for eight seconds; images were captured using a digital SLR camera under standard illumination. (B) CIE L\*, a\*, and b\* values of the  $0.017 \times 0.025$  inch and  $0.019 \times 0.025$  inch unheated SS wire specimens and specimens heated at power settings of 2, 4, 6, and 8 for eight seconds; images were captured using a digital SLR camera under standard illumination. (B) CIE L\*, a\*, and b\* values of the  $0.017 \times 0.025$  inch and  $0.019 \times 0.025$  inch unheated SS wire specimens and specimens heated at power settings of 2, 4, 6, and 8 for eight seconds under standard illumination. (C)  $\triangle$ E values (colour difference) between any two groups of unheated and heated  $0.019 \times 0.025$  inch SS wire specimens. (D)  $\triangle$ E values (colour difference) between any two groups of unheated and heated  $0.019 \times 0.025$ -inch SS wire specimens.



Figure 7. (A) Schematic showing the intercanine width and the intermolar width. (B) Intercanine width and intermolar width of  $0.017 \times 0.025$  inch unheated SS wire specimens and specimens heated at power settings of 2, 4, 6, and 8 for eight seconds. (C) Intercanine width and intermolar width of  $0.019 \times 0.025$  inch unheated SS wire specimens and specimens heated at power settings of 2, 4, 6, and 8 for eight seconds. Bars with dissimilar letters indicate significantly different values (p < 0.05). Each value is presented as the mean  $\pm$  SD (N = 3).

same type. In the present study, the flexural modulus of the 0.017  $\times$  0.025 inch SS and 0.019  $\times$  0.025 inch SS wires was significantly increased after eight seconds of heat treatment at power settings of 4, 6, and 8. Specifically, under the same amount of force, the heattreated SS wires showed small elastic deformation and greater stiffness. Therefore, large unheated arch wires were able to be replaced with the small heat-treated arch wires. This protocol reduces the diameter of the arch wire and, further, reduces the sliding resistance while maintaining the required rigidity of the wire.

When SS is exposed to high temperatures, such as during heat treatment or welding, discolourations are caused by oxidation of the steel.<sup>31</sup> The SEM results showed no obvious pitting on the surface of the SS wires after heat treatment, but the number of surface impurities increased, and the surface unevenness of the arch wire became more noticeable. However, the COF, a constant that is closely related to the superficial characteristics of a material, increased in the 0.017 × 0.025 inch SS wire at power settings of 6 and 8 and in the 0.019 × 0.025 inch SS wire at a power setting of 8, indicating an increase in the surface roughness following the increase in the heat treatment power setting, and an increased COF may lead to an increased frictional force.<sup>32</sup>

## Cytotoxicity

SS does not corrode easily because of an electrochemically formed passive film, which shields the material from aggressive ions in the air and offers protection. However, when SS is heat-treated, surface oxidation can occur, and an uneven oxide film may cause localised corrosion. Oh et al.<sup>33</sup> investigated the cytotoxicity of four types of SS wires (Remanium, Permachrome, Colboloy and Orthos) of crosssectional areas of  $0.41 \times 0.56$  mm before and after heat treatment in a vacuum, air, or argon environment and subsequent cooling in either a furnace or a water bath. Both the control and experimental groups showed low toxicity, but the air heat treatment group showed a slightly higher reaction compared with the other groups.

The findings of Oh et al.<sup>33</sup> are similar to the present experimental results. The cytotoxicity findings, based on the relative increase in the cell growth rate, revealed only slight cytotoxicity compared with the untreated group when the  $0.019 \times 0.025$  inch SS wire was heated at a power setting of 8 for eight seconds, and the other groups showed no significant cytotoxicity. However, the heat treatment of an SS wire in air reduced the proliferation of human gingival fibroblasts as the power setting increased, indicating that heat treatment of the SS wire at a higher power setting increases the concentration of eluted ions, thereby affecting the growth of gingival fibroblasts.

## XRD and austenitic transformation

Martensitic transformation occurs when austenitic SS undergoes tensile plastic deformation or cold rolling. This phase change weakens the mechanical properties of the arch wire by significantly reducing the elastic modulus and ductility.<sup>34</sup> Therefore, the ability to measure and control the relative proportions of the two phases is essential to optimise the force transfer properties of the wire for use in clinical practice and material research.<sup>35</sup>

Appropriate heat treatment requires precise control over the temperature, the time of treatment at a particular temperature and the cooling rate.<sup>36</sup> In the present experiment, the heated material was air cooled, and the phase product of heat treatment and annealing of the SS wire was related to the annealing temperature and the cooling rate after annealing. Air-cooling is a rapid cooling method, which may explain the reduced austenitic content observed after heat treatment and therefore an increased martensitic content. Since the goal of heat treatment is to reduce the martensitic content to the greatest extent possible and therefore improve the mechanical properties, other cooling methods should be considered to reduce the phase transformation after heat treatment.

# Heat-induced changes in colour as a heat treatment indicator

Newly ground or polished steel forms an oxide layer when heated.<sup>37</sup> The heat-induced colour change can be used to determine the final properties of the tempered steel. Very hard tools are often adjusted in the light yellow to dark yellow range, while springs are usually adjusted to blue.<sup>38,39</sup> For orthodontic SS arch wires, some researchers recommend heating until the arch wire changes from silver to reddish brown<sup>14</sup> in order to achieve the desired mechanical properties. In the present study, the flexural modulus was used as an indicator of the mechanical properties of the SS wires, and brownish-yellow and blue SS wires showed higher flexural moduli. Therefore, it is believed that the colour of an SS wire surface can be used as an indicator of the heat treatment temperature, but a single colour should not be used as an indicator of appropriate mechanical properties.

The colour information acquired by a digital camera depends on the device incorporating the actual colour information, which is usually presented in the RGB colour space and differs between devices.<sup>40-42</sup> In the present study, the device-dependent colour images from a digital camera were converted to a standard device-independent colour space (i.e., CIELAB) and the calculated colour difference was measured by  $\triangle E$ values. Previous research indicates that  $\triangle E$  values less than 1.0 are impossible for the eye to detect. According to related studies,  $\triangle E$  values less than 2.5 are not visible to the casual user of an image captured in the real world.43,44 Based on the present experimental results for the 0.017  $\times$  0.025 inch SS and 0.019  $\times$ 0.025 inch SS wires heated at different power settings, the  $\triangle E$  value of any two groups was greater than 2.5, indicating that the change in the colour of the arch wire with each heat treatment was obvious in this experimental design, and each treatment resulted in a colour change that was easily distinguished by the human eve.

## Arch width adjustment

The arch wire determines the future arch shape, and the teeth move according to the created form.<sup>45</sup> The use of pre-adjusted orthodontic arch wires simplifies the form of the arch but does not eliminate the need for personalised orthodontic arch wires.<sup>46</sup> Therefore, in clinical practice, the shape of the arch wire should be appropriately adjusted according to each patient's dental arch. For the heated  $0.017 \times 0.025$  inch SS and  $0.019 \times 0.025$  inch SS wires, the widths between the molars and canines only increased at power settings of 2 and/or 4, while at power settings of 6 and 8, the widths between the molars and canines were not different from the untreated group, indicating that the SS arch wire had significantly expanded immediately after the heat treatment. However, the arch wire returned and stabilised at its original shape and width. Therefore, under the conditions used to obtain the desired mechanical properties, the heat treatment did not increase the width of the arch wire, and the arch shape did not require adjustment after the heat treatment.

However, the present study still presented limitations. Firstly, in vitro experiments do not completely simulate the clinical situation. In practical applications, the SS wire is placed in the mouth, is coated in saliva, and the effects of food and the adhesion of bacteria alter the performance of the wire. Whether the thermal colour formed on the surface of the SS wire after heat treatment has an effect on the corrosion property of the SS wire and the adhesion of bacteria was not assessed. Secondly, whether the adverse effects of heat treatment on COF and cell proliferation are eliminated or removed by removing the oxide layer on the surface was not evaluated. These questions will require an in-depth study in the future.

## Conclusions

Based on the results of the present experiments, the COF, flexural modulus and austenitic content of the heat-treated SS arch wires increased, which are properties that reduce sliding resistance during space closure. The wire colour gradually changed from silver to brownish-yellow and blue, and the maximum flexural modulus was obtained for wires with a brownish-yellow colour, which can be used as an indicator to determine heat treatment conditions. Heat treatment of an SS wire in air reduced the proliferation of human gingival fibroblasts but did not produce cytotoxic effects. The use of electric heating equipment for the heat treatment of orthodontic arch wires is an effective and feasible method.

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