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博 士 論 文

Chemical modification to suppress metal ions elution of
dental orthodontic stainless steel wire surface

(矯正歯科用ステンレススチールワイヤーへの
イオン溶出抑制を目的とした表面化学修飾)

令和2年3月申請

北海道大学

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1. Abstract

In this study, we investigated the suppression of metal ion elution on dental orthodontic stainless steel wire. Metal ion elution using Cr and Ni can potentially cause a metal allergy. To suppress metal ion elution from stainless steel wire, three types of surface chemical modifications were carried out. The obtained wire was immersed into several aqueous solutions, and then the eluted ions were determined using coupled plasma mass spectrometry (ICP-MS). By both electropolymerization and self-assembly monolayer formation, the ion elution was effectively reduced. Further, the stainless steel surface was modified using a polyamine, renowned for its antibacterial properties. This surface modification indicated a metal ion suppression effect. These results suggested that these surface modifications succeeded in suppressing the metal ions.

2. Introduction

Chemical modification, such as organic monolayer formation, is one of the most simple and useful methods for the addition of multiple functions to the metal and alloy surface¹⁻⁴⁾. Various researchers have conducted investigations related to several fields, such as nanoelectronics⁵⁾, optoelectronics⁶⁾, chemical and biochemical sensing⁷⁾, bioengineering⁸⁾, life sciences⁹⁾, and biomedical applications¹⁰⁾. The recent development of medical technology has required not only new function, such as mechanical, electrical, and optical, but also biocompatibility. Stainless steel is a traditional and useful material even in these fields. However, stainless steel is “biotolerant”, as opposed to “bioinert” or “bioactive”. When used for a long time in a biological environment, metal ions can be eluted. Some metal ions contained in stainless steel, such as Cr, Ni, and Pb, can cause metal allergies¹¹⁻¹⁴⁾. In the dental field, metal allergies constitute a delayed type of allergy that can be classified as type IV¹⁵⁾. They are considered to be caused by incomplete antigens formed by the binding of the Ni and Cr ions with proteins in the oral environment. Thus, the suppression of their elution may reduce the risk of metal allergies.

In this study, we initially investigated the chemical modifications on the surface of the dental orthodontic stainless steel wires and subsequently evaluated their suppression effects on metal ion elution. For the surface modification, we investigated the electropolymerization and self-assembly monolayer (SAM) formation on dental stainless steel wire. Then the obtained wires were immersed into acidic solutions (hydrochloric acid [HCl], phosphoric acid [H₃PO₄], and lactic acid) for one or four weeks. To estimate the suppression of the metal ion elution, the amounts of Cr and Ni

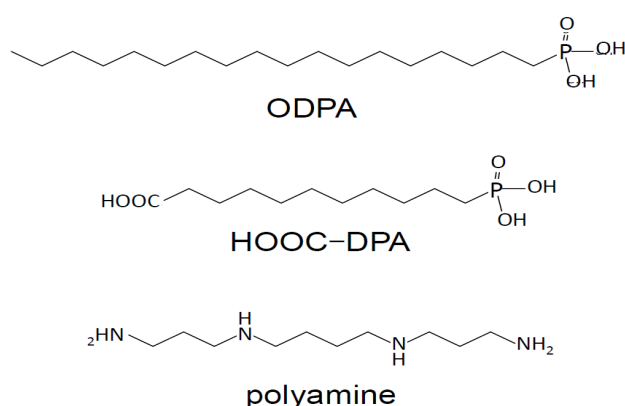
ions in the supernatant were determined. In addition, we prepared a polyamine-tethered SAM on the wire using the condensation reaction. Although the polyamine derivatives are renowned to possess antibiological properties, surface modification with polyamines can provide bifunctional properties^{20, 21)}, decrease the risk of metal allergies, and provide the metal with antibiological properties.

3. Materials and methods

3.1 Materials

The dental orthodontic stainless steel wire (TRU-CHROME, 0.016" × 0.022") was purchased from JM Ortho Co., (Tokyo, Japan). Pyrrole and sodium dodecylbenzenesulfonate were purchased from Sigma-Aldrich (St. Louis, MO, USA). Octadecylphosphonic acid (hereafter denoted as ODPA) was provided by Miyoshi Oil & Fat Co. Ltd. (Tokyo, Japan). 10-carboxydecylphosphonic acid (hereafter denoted as HOOC-DPA) was purchased from Wako Inc. (Tokyo, Japan).

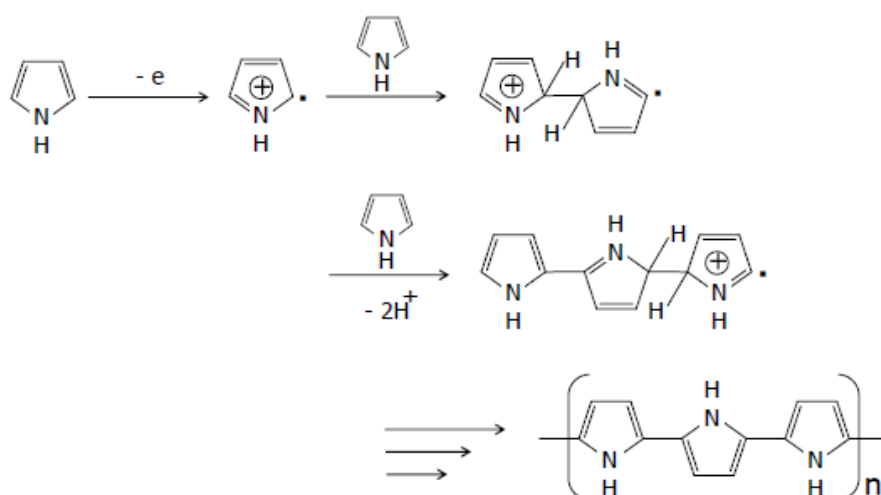
N,N'-Bis(3-aminopropyl)tetramethylenediamine phosphate hexahydrate (hereafter denoted as polyamine) was purchased from Tokyo Chemical Industry Co., Ltd. (Tokyo, Japan). 4-(4,6-Dimethoxy-1,3,5-triazin-2-yl)-4-methylmorpholinium chloride (hereafter, denoted as DMT-MM) was purchased from Sigma-Aldrich (St. Louis, MO, USA). All the chemicals were used without any further purification.



Scheme 1. Chemical structures of alkyl phosphates and the polyamine.

3.2 Electropolymerisation

Stainless steel wire was cut into 80 mm length. Before surface modification, wires were washed with distilled water and acetone. Electropolymerisation was performed under constant voltage in an electrochemical cell with a volume of 50 mL at room temperature. The solution used for electropolymerisation was 10 mol/L sodium dodecylbenzenesulfonate containing 50 mmol/L Py monomer. The counter electrode was platinum foil. The voltage was controlled as 1, 3 and 10 V by a DC power supply with 10 min. to 60 min. as a reaction time. The illustrated reaction scheme was indicated in Scheme 2.



Scheme 2. Illustrated scheme of electropolymerization.

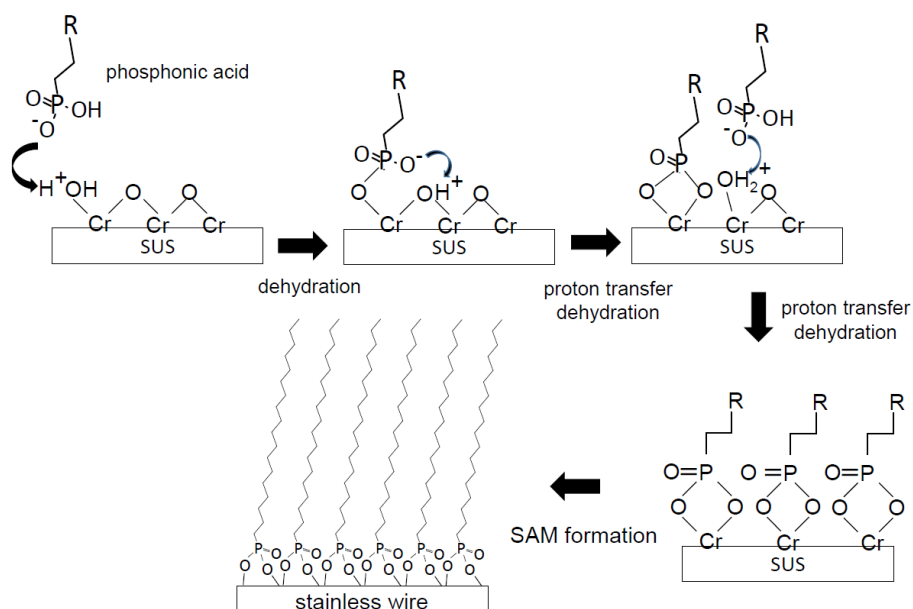
3.3 Determination of metal ion elution via polypyrrole-modified dental wire

The surface-modified dental wires were immersed into 12 mL of several aqueous solutions, such as 50 mM HCl, 1 M acetic acid, and saline. They were kept in 37 °C for 7 days under shaker. After immersion, wires were removed then obtained supernatant solutions were filtrated with milli-pour filter (Millex-GS, Millipore, Feltham, UK). The concentration of eluted metal ions were determined using an inductively coupled plasma–mass spectrometry (ICP–MS) (8800 ICP-QQQ, Agilent technologies, Tokyo,

Japan). For comparison results by uniform surface area, each obtained concentrations were normalized by length of the wire.

3.4 Self-assemble monolayer formation

The dental orthodontic wires were cut to 80 mm each in case of length, and they were washed using a natural detergent solution, acetone, and methanol for 10 min while being subjected to sonication. After rinsing using distilled water, the wires were immersed into 100 mL of 10 mM alkyl phosphonic acid/dehydrated ethanol and stirred for 2 h, followed by drying at 120 °C for two days. This procedure slightly modified in Marder's condition²²⁾. The illustrated reaction scheme was indicated in Scheme 3.

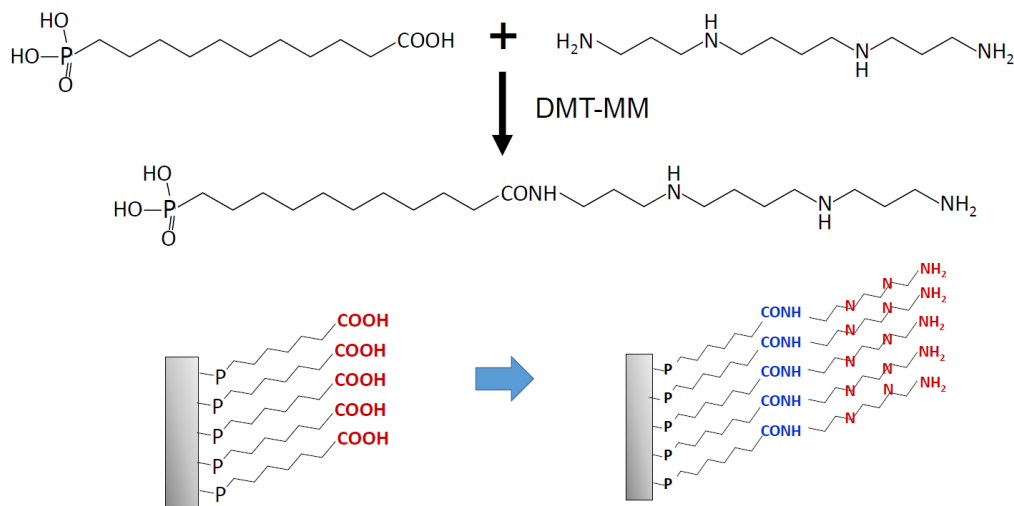


Scheme 3. Scheme of SAM formation.

3.5 Polyamine modification of the stainless steel surface

To tether the polyamine on the stainless steel surface, the aforementioned cleaned bare stainless steel was initially modified with using HOOC–DPA. The obtained wire was subsequently modified using –COOH on the end of the SAM and was subsequently immersed into a 10 mM polyamine/dehydrated ethanol solution and stirred for 2 h. Further, 1.2 eq. of DMT–MM was added to the reaction mixture to enhance the amide bond formation. The procedure was in accordance with our previous conditions²³⁾ even

though some minor modifications were introduced. The illustrated reaction scheme was indicated in Scheme 4.



Scheme 4. Scheme of polyamine-tethered SAM formation.

3.6 Characterization of the obtained SAM

The surface wettability was observed to identify SAM formation on the stainless steel surface. First, 10 μL of distilled water was dropped on the surface at 25 $^{\circ}\text{C}$. The contact angle was recorded using a VHD-3000 digital microscope (Keyence, Tokyo, Japan). The lens portion of the digital microscope was rotated by 90 degrees, and the sample which distilled water was dropped was observed from the horizontal direction. Further, the contact angle was calculated from the obtained image using the attached analysis program. The thickness of the obtained SAM was estimated using a spectroscopic ellipsometer (DHA-F, Mizojiri Optics, Tokyo, Japan). To determine the amide bond formation, a micro-Raman spectroscopic system (inVia Reflex; Renishaw K.K., Tokyo, Japan) was used.

3.7 Determination of metal ion elution via SAM-modified dental wire

To estimate the suppression effect of metal ion elution by SAM formation, the obtained wires coated with SAM were immersed into several aqueous solutions (HCl, H₃PO₄, lactic acid, and saline) for one or four weeks at 37 $^{\circ}\text{C}$ while being stirred. Subsequently,

the amount of eluted metal ions was determined using inductively coupled plasma–mass spectrometry (ICP–MS) (8800 ICP–QQQ, Agilent technologies, Tokyo, Japan).

4. Results and discussion

4.1 Polypyrrole layer formation

Polypyrrole layers were formed on dental stainless wire by electropolymerization. Obtained layers strongly depended upon the polymerization condition. Figure 1 shows digital microscopic images of cross-section of PPy-wires. The thickness of obtained PPy layer were different from the electropolymerization's condition. With increase the applied voltage, the thickness of PPy layer increases. The value is less than 1 μm at 5 V (Fig. 1a), 5–10 μm at 10 V (Fig. 1b), and ca. 20 μm at 20 V (Fig. 1c). Though thickness of the layer increases as the applied voltage increases, shape of the obtained layer becomes random.

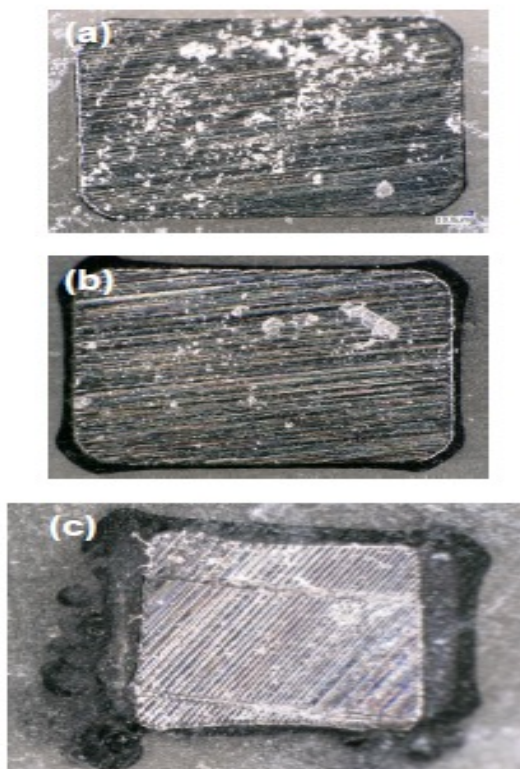


Figure1. Microscope images of cross-section of polypyrrole-coated dental wire.

To understand the structure details, SEM images of the layers under different electropolymerization conditions were observed (Figure 2). Under low-voltage conditions, submicron-sized, spherical particles sparsely appeared on the surface (as shown in Fig 2a - 2d). When the electropolymerization was performed under 1 V, the polymer layer nonhomogeneously covered the wire surface and was easily removed from the surface (i.e., the coating was defective). These particles then agglomerated in a straight line, and the number of micro-sized particles increased (Fig 2e and 2f). When the reaction time reached 60 min, the layer comprised aggregates of submicron-sized particles (Fig 2g). In contrast, homogeneous and stable layers were obtained when the applied voltage was increased. The layers polymerized at 10 V had a different surface (Fig 2h), comprising aggregates, even after 10 min.

To understand the structure details, SEM images of the layers were observed. When the electropolymerization was carried out with under 1 V of applied voltage, homogeneous polymer layer was not obtained. The polymer layer covered wire surface unhomogeneously. Sometimes the layer was easily removed from the surface. In other words, the layer formed a defective coating. In contrast, homogeneous and stable layers were obtained with increased applied voltage. Figure 2 shows SEM images of the obtained PPy layers' surface with different polymerization conditions. Under low voltage conditions, spherical particles, which consisted of small particles with submicrom size, appeared on the surface sparsely (as shown in Fig 2a - 2d). Then they agglomerated on a straight line and the number of the micro-sized particles increased (as shown in Fig 2e and 2f). As the reaction time reached 60 min., the layer consisted of aggregates of the submicron particles (as shown in Fig 2g). In contrast, the layers polymerized at 10 V indicated a different surface (as shown in Fig 2h). They consisted of the aggregates even after a 10 min. reaction.

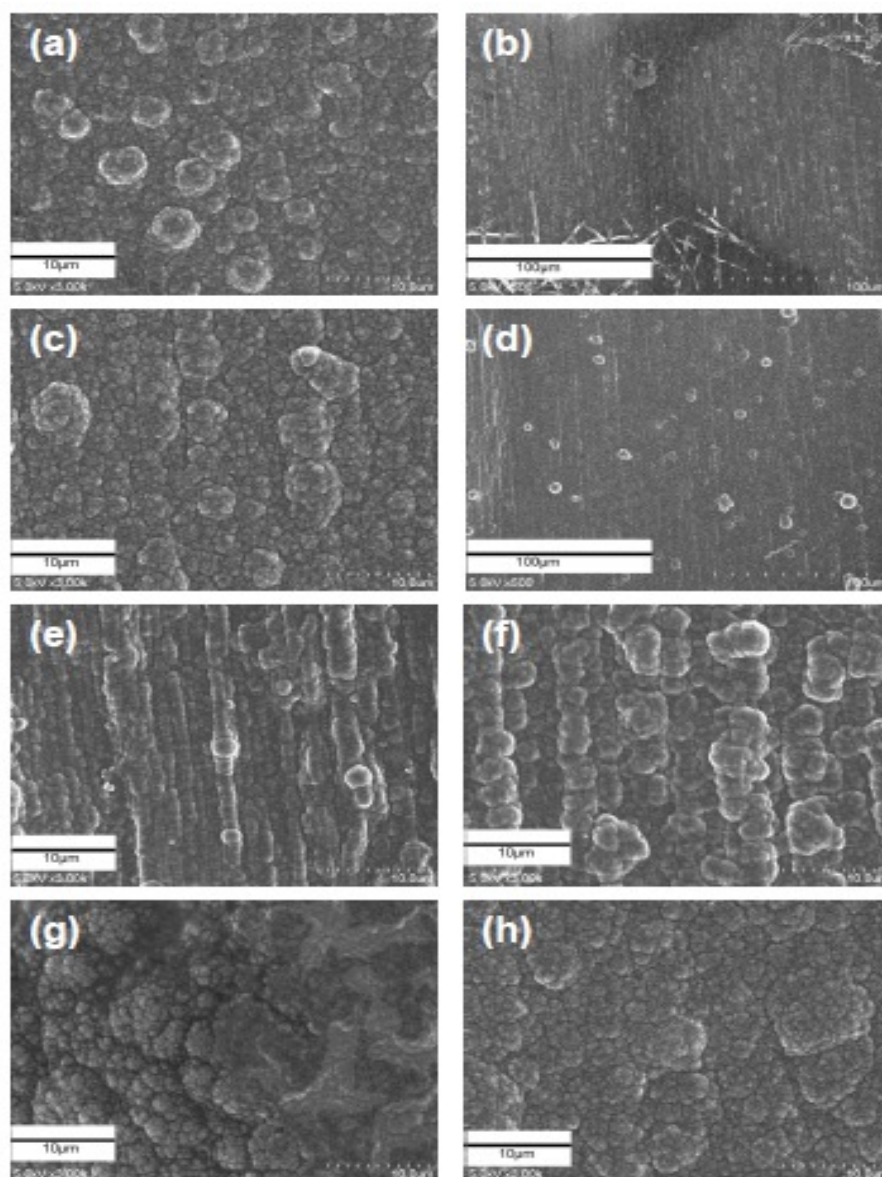


Figure 2. SEM images of polypyrrole-coated dental wire: (a) 1V, 10 min., (b) 1V, 10 min. (low mag.), (c) 3V, 10 min., (d) 3V 10 min. (low mag.), (e) 1V, 30 min., (f) 3V, 30 min., (g) 3V, 60 min., (h) 10V, 10 min.

4.2 Suppression of metal ion elution via polypyrrole-modified dental wire

Figure 3 shows typical SEM images of dental stainless wire's surface. When the wires were immersed into acid solution, pitting corrosion were carried out as shown in Figure 3b. With increasing the acid concentration, the corrosion of wire surface proceeded more severe. To estimate the suppression effect of metal ion elution, dental wires which were modified the surface by organic layer were immersed into several aqueous solutions.

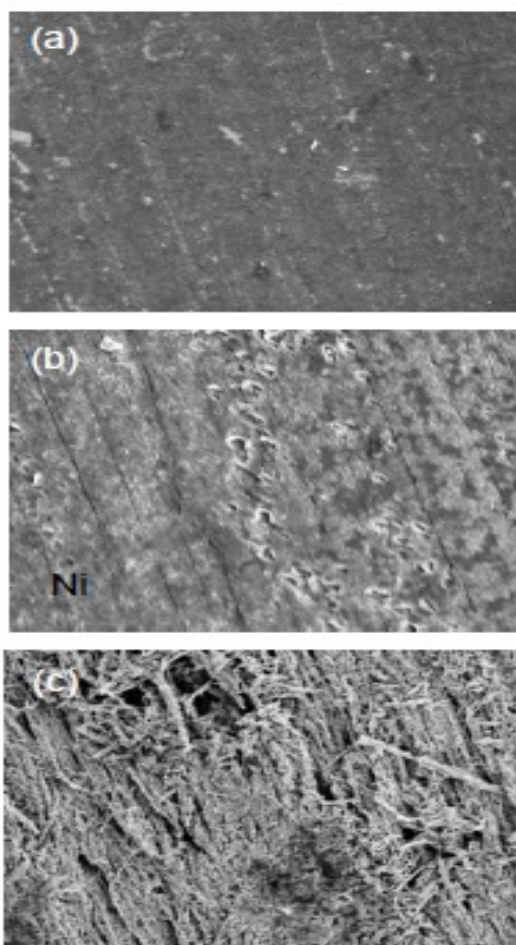


Figure 3. SEM images of surface of dental wire (before (a), and after HCl immersion (b) 0.2 M, (c) 0.5 M).

Figure 4 shows concentration of eluted metal ions from polypyrrole-coated dental wire immersed into 50 mM HCl aq., 1 M acetic acid, and saline solution. With

PPy-modification, amount of eluted Cr and Ni ions were suppressed compared with non-coated ones (control). Only eluted Cr ions from saline were detected almost same amount as that of control ones. To estimate PPy-layer coated wire, which were prepared with different conditions, were immersed into 1 M lactic acid solution. With increase applied voltage or polymerization time, amount of eluted ions decrease (as shown in Figure 5).

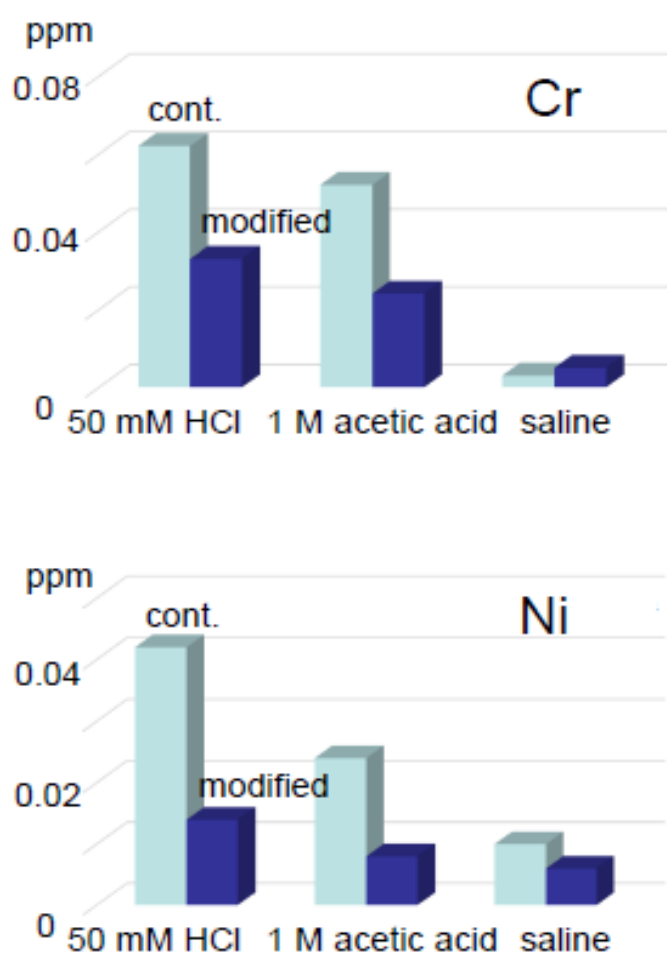


Figure 4. Concentration of eluted metal ions from polypyrrole-modified dental wire, (top): Cr ions, (bottom): Ni ions.

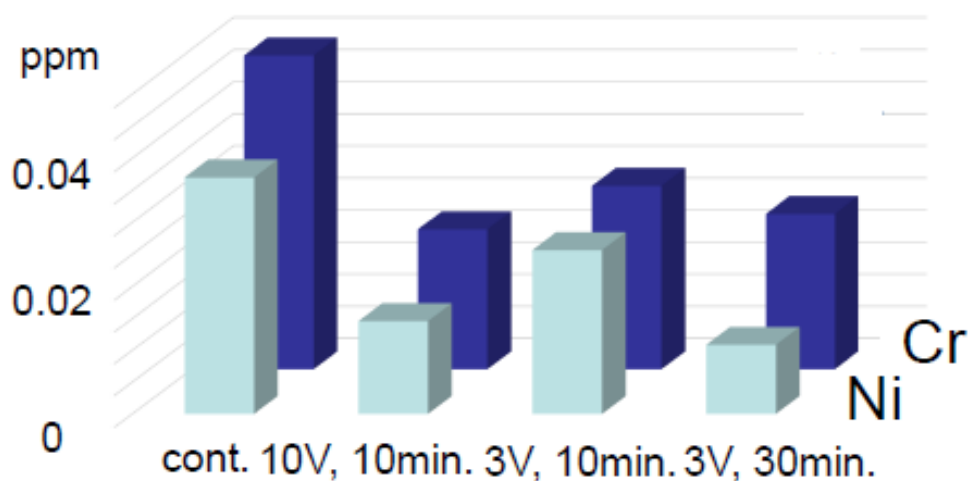


Figure 5. Concentration of eluted metal ions from polypyrrole-modified dental wire with different polymerization condition.

4.3 Wettability of the SAM-modified stainless steel surface

Figure 6 depicts the contact angles of the stainless steel substrate before and after SAM formation. Depending on the modifications, the obtained contact angle was verified. When the surface was modified using a long-chain alkyl phosphonate, the contact angle increased from 52 ± 4 to 65 ± 4 [Figure 1(b)]. However, the contact angle decreased when a carboxyl group was added to the end of alkyl phosphonate [as depicted in Fig. 3. The contact angle increased again when polyamine was tethered to the carboxyl-terminated surface.

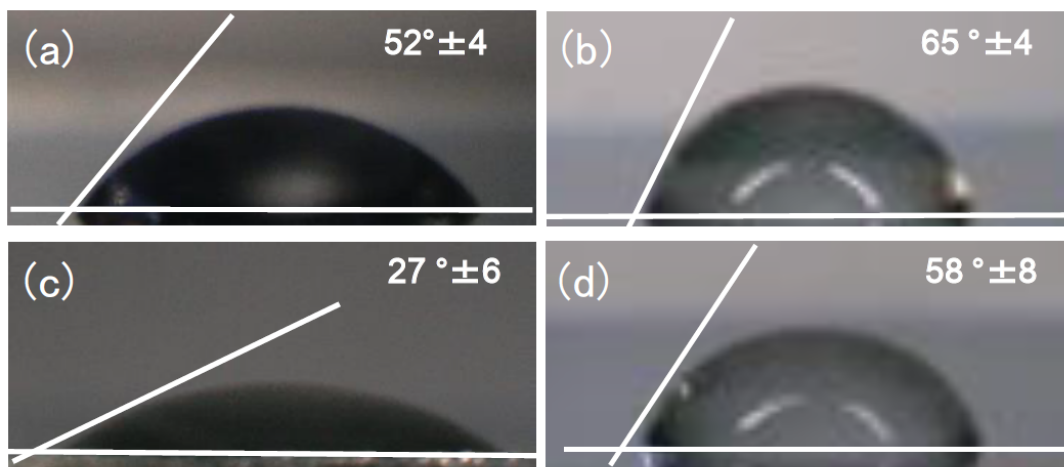


Figure 6. Contact angle of the water–stainless steel surface: (a) control, (b) ODPa-SAM-modified samples. (c) HOOC-DPA-SAM-modified samples, and (d) polyamine-tethered HOOC-DPA-SAM-modified samples.

4.4 Suppression of metal ion elution via SAM-modified dental wire

The orthodontic dental stainless steel wire was immersed into several acidic solution to estimate the suppression effect of the metal ion elution. After one or four weeks of immersion, the amounts of Cr and Ni ions in the supernatant were determined using ICP–MS. As depicted in Fig. 7, the amounts of Cr and Ni ions immersed in H_3PO_4 and lactic acid significantly decreased upon ODPa–SAM formation. When the bare wire was immersed in 0.1 M of phosphoric acid for one week, 0.06 ppm of Cr ions was eluted per 10 mm of the wire. However, the surface modification suppressed the elution by less than 0.03 ppm. In case of 0.1 M of lactic acid, the eluted amount was low and was not significantly different. After four weeks of immersion, the modification suppressed the amount from 0.06 to 0.02 ppm. In case of Ni ion elution, the behavior was observed to be similar to that of the Cr ions. However, the formation of SAM did not effectively inhibit elution from the sample immersed in aq. HCl. One reason may be that the Cl^- ions are small and can diffuse though the SAM film, directly affecting the wire’s surface.

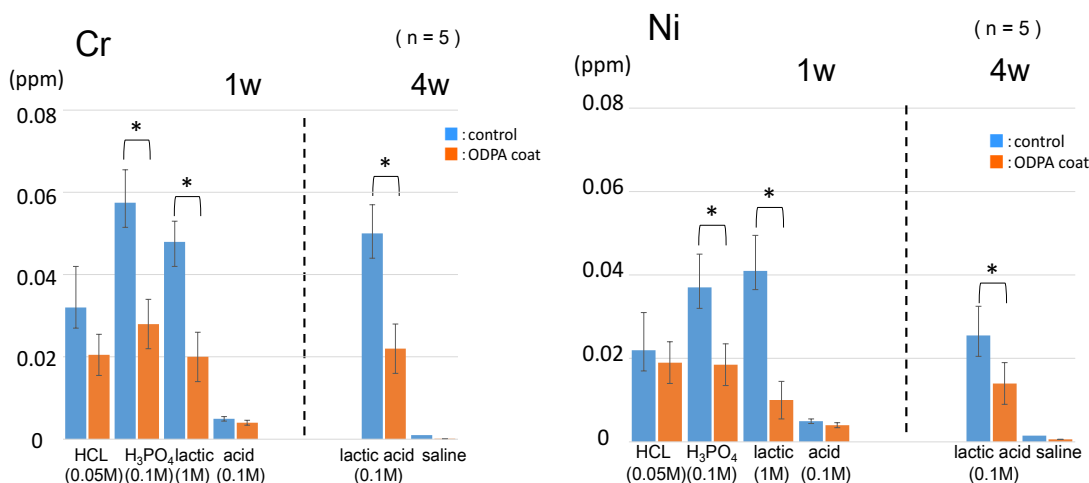


Figure 7. Concentration of eluted metal ions from the ODPA-modified dental wire. (a) Cr ions. (b) Ni ions.

4.5 Surface modification using polyamine

To provide antibacterial properties, polyamine was modified on the stainless steel surface. As presented in Scheme 4, the surface was modified using a carboxyl group at the end of the long chain phosphate (HOOC–DPA), and the polyamine reacted with the carboxyl group using a condensation agent. The formation of SAM was characterized using contact angle measurement and Raman spectroscopy.

As depicted in Fig. 6, the contact angle decreased with the formation of the long alkyl-chain phosphonate SAM that contained a carboxyl group at the end [Fig. 6(c)]. Then, the angle increased again after the condensation reaction [Fig. 6(d)]. This result suggests that the hydrophilic surface that contained the carboxyl group was converted into a less hydrophilic one because of polyamine conjugation. Generally, amines or amides exhibit lower hydrophilicity when compared with that exhibited by the carboxyl groups. In fact, the thickness of the layer increased from 1.7 ± 1.0 to 2.9 ± 0.9 nm after the polyamine condensation reaction. The thicknesses were estimated using spectroscopic ellipsometry. In addition, the Raman spectrum of the surface indicated an increase in the peaks at approximately $1,300\text{ cm}^{-1}$ (assigned to N–H) and a decrease in the peaks at approximately $3,200$ and $3,400\text{ cm}^{-1}$ (assigned to –COOH). These results revealed a decrease in the number of –COOH groups and the appearance of N–H (–NH₂ and N–H). Therefore, some –COOH groups, which were on the end of the hydrophilic SAM, were converted into polyamine upon amide bond formation, as depicted in Fig. 8.

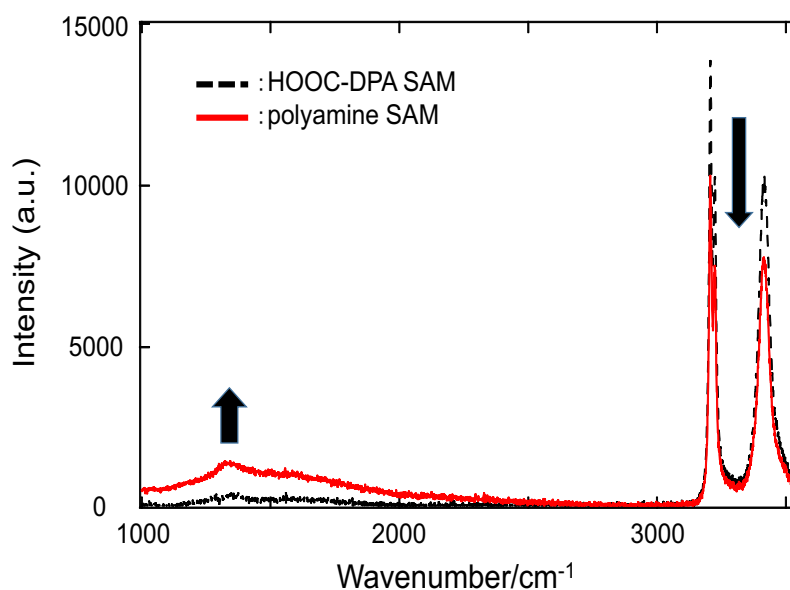


Figure 8. Raman spectra showing the eluted metal ion polyamine–tethered reaction on the stainless steel surface.

These results indicated the successful polyamine modification on the carboxyl-terminated surface. The obtained surface–modified stainless steel wire was also immersed in 1 M of lactic acid for one week and 0.1 M of lactic acid for four weeks. As depicted in Fig. 9, the obtained polyamine-modified SAM indicated the suppression of metal ion elution. Upon polyamine modification, the amounts of both the Cr and Ni ions significantly decreased. The results indicated that polyamine-modified SAM significant suppressed both of metal ion's elution as same as those of ODPA-SAM.

Generally, polyamines are known to possess antibacterial properties. Therefore, the modification of stainless steel wires using a polyamine can provide metal ion suppression and antibacterial properties that may be useful in not only dental but also all medical fields.

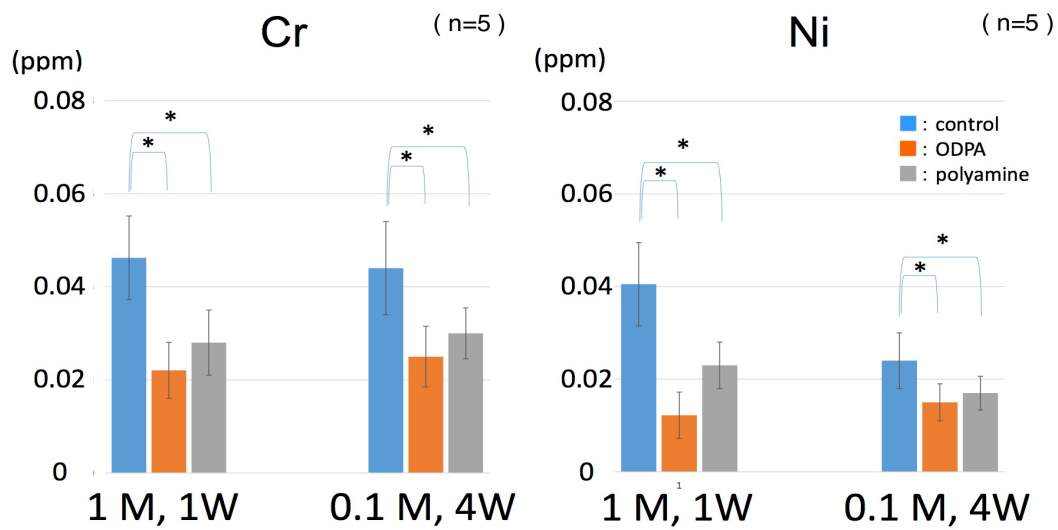
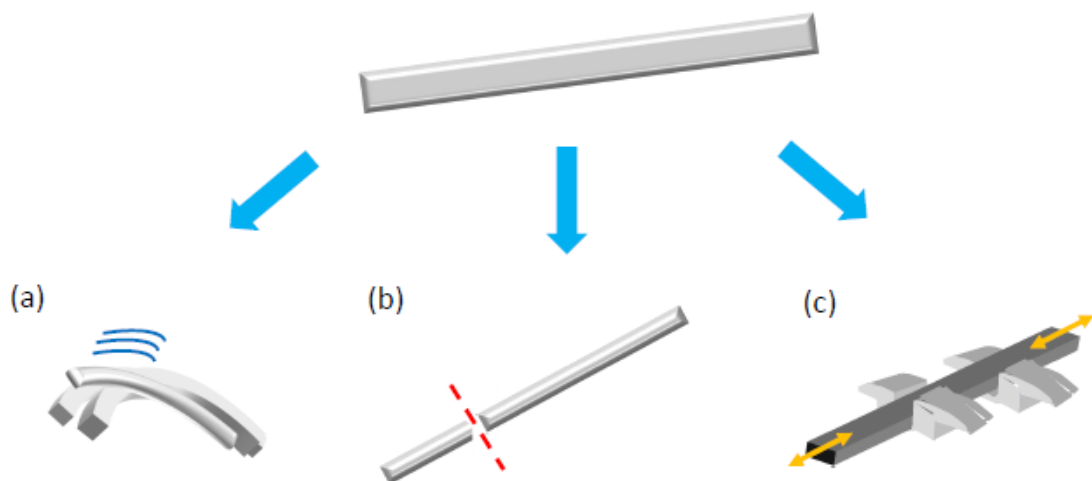


Figure 9. Concentration of eluted metal ions from the polyamine-modified dental wire. 1M lactic acid for one week and 0.1M lactic acid for four weeks, left: Cr ions, right: Ni ions.

In general, dental orthodontic wires undergo several processing adjustments such as vent, end-cut, and slide in multi-bracket, etc. (as shown in Scheme 5). Thus, the surface modification may affect the fatigue properties of the wire due to these mechanical stresses. For dental use, we should investigate these properties in future work.



Scheme 5. Illustrated scheme of typical intra-oral adjustments of orthodontic wire processing (a) bending, (b) cutting, and (c) abrasion (passing dental orthodontic bracket).

5. Conclusions

In this study, we investigated three types of surface modifications, that is an electro polymerization, a long alkyl chain and a polyamine monolayer, on orthodontic dental stainless steel wires. Subsequently we evaluated the suppression of metal ion elution formation on the surface of the wires because metal ion elution with elements Cr and Ni has the potential to cause metal allergies. These surface modifications were successful, and the obtained thin layer that suppressed the elution of Cr and Ni ions from the dental wire. In general, polyamines are known for their antibacterial properties. Therefore, these surface modifications can be used for bifunctional materials in not only the dental but also other medical fields.

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