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Original Research

Chemical, Morphological and Bacterial Adhesion Analysis of Orthodontic Wires Composed of Different Metallic Alloys

Análise Química, Morfológica e de Adesão Bacteriana de Fios Ortodônticos Compostos por Diferentes Ligas Metálicas
Análisis Químico, Morfológico y de Adherencia Bacteriana de Alambres de Ortodoncia Compuestos por Diferentes Aleaciones Metálicas

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Abstract

The purpose of this study was to evaluate the chemical, morphological and bacterial adhesion characteristics of orthodontic arches composed of different metal alloys. The wire segments (n=10) were allocated to the following groups: G1) Tru-Chrome- Rock Mountain Steel Wire (Colorado-USA); G2) NiTi- Rock Mountain Wire (Colorado-USA); G3) TiMb Rock Mountain Wire (Colored-USA); G4) NbTi Gummetal- Rock Mountain Wire (Colorado-USA). The orthodontic arches were segmented (20mm) and sterilized by means of ultraviolet light. Using Confocal Laser Microscopy (CLM), the roughness of archwires were investigated. The metallic alloys composition was analyzed by means of Scanning Electron Microscopy/Energy-dispersive Spectroscopy (SEM/EDS). The *S. mutans* biofilm growth was performed on the arches and analyzed by means of SEM and Spectrophotometry. Surface roughness and Spectrophotometry data were submitted to one-way ANOVA, followed by Tukey's test (α=0.05) and SEM/EDS data obtained by exploratory analysis. The NbTi arches showed higher surface roughness when compared to the other groups, followed by the NiTi and TiMb arches and the steel arches group ($p<0.05$). It was observed a lower adherence of *S. mutans* biofilm in steel arches when compared to the other groups. The absorbance results showed higher biofilm formation for the NbTi group, followed by Steel, NiTi and TiMb. ($p<0.05$). The results of EDS confirm the compositions proposed by the manufacturer. It was concluded that the alloy type in orthodontic arches has an effect on surface roughness. The chemical and morphological characteristics of the arches are related to the adhesion of S. *mutans* biofilm.

Descriptors: Alloys; Orthodontics; *Streptococcus mutans*; Wire.

Resumo

O objetivo deste estudo foi avaliar as características químicas, morfológicas e de adesão bacteriana de arcos ortodônticos compostos por diferentes ligas metálicas. Os segmentos de fio (n=10) foram alocados nos seguintes grupos: G1) Fio de Aço Tru-Chrome Rock Mountain (Colorado-EUA); G2) Fio de NiTi Rock Mountain (Colorado-EUA); G3) Fio de TiMb Rock Mountain (Colorado-EUA); G4) Fio de NbTi Gummetal Rock Mountain (Colorado-EUA). Os arcos ortodônticos foram segmentados (20mm) e esterilizados por meio de luz ultravioleta. Utilizando a Microscopia Confocal a Laser (CLM), a rugosidade dos arcos foi investigada. A composição das ligas metálicas foi analisada por Microscopia Eletrônica de Varredura/Espectroscopia de Energia Dispersiva (SEM/EDS). O crescimento do biofilme de *S. mutans* foi realizado nos arcos e analisado por meio de SEM e espectrofotometria. Os dados de rugosidade superficial e espectrofotometria foram submetidos a ANOVA unifatorial, seguida pelo teste de Tukey (α=0,05) e os dados de SEM/EDS foram obtidos por meio de análise exploratória. Os arcos NbTi apresentaram maior rugosidade superficial em comparação com os outros grupos, seguidos pelos arcos NiTi e TiMb e pelo grupo de arcos de aço ($p<0,05$). Foi observada uma menor aderência do biofilme de *S. mutans* nos arcos de aço em comparação com os outros grupos. Os resultados de absorvância mostraram uma maior formação de biofilme para o grupo NbTi, seguido por Aço, NiTi e TiMb ($p<0,05$). Os resultados de EDS confirmam as composições propostas pelo fabricante. Concluiu-se que o tipo de liga em arcos ortodônticos tem um efeito na rugosidade superficial. As características químicas e morfológicas dos arcos estão relacionadas com a adesão do biofilme de *S. mutans.*

Descritores: Ligas; Ortodontia; *Streptococcus mutans*; Fio.

Resumen

El propósito de este estudio fue evaluar las características químicas, morfológicas y de adhesión bacteriana de los arcos ortodónticos compuestos por diferentes aleaciones metálicas.: Los segmentos de alambre (n=10) se asignaron a los siguientes grupos: G1) Tru-Chrome- Rock Mountain Steel Wire (Colordo-EE. UU.); G2) NiTi- Rock Mountain Wire (Colorado-EE. UU.); G3) TiMb Rock Mountain Wire (Colorado-EE. UU.); G4) NbTi Gummetal- Rock Mountain Wire (Colorado-EE. UU.). Los arcos ortodónticos se segmentaron (20 mm) y se esterilizaron mediante luz ultravioleta. Utilizando la Microscopía Confocal de Láser (CLM), se investigó la rugosidad de los arcos. La composición de las aleaciones metálicas se analizó mediante Microscopía Electrónica de Barrido/Espectroscopía de Dispersión de Energía (SEM/EDS). Se realizó el crecimiento del biofilm de *S. mutans* en los arcos y se analizó mediante SEM y espectrofotometría. Los datos de rugosidad superficial y espectrofotometría se sometieron a un ANOVA de una vía, seguido por la prueba de Tukey (α=0.05) y los datos de SEM/EDS se obtuvieron mediante análisis exploratorio. Los arcos NbTi mostraron una mayor rugosidad superficial en comparación con los otros grupos, seguidos por los arcos NiTi y TiMb y el grupo de arcos de acero ($p<0.05$). Se observó una menor adherencia del biofilm de *S. mutans* en los arcos de acero en comparación con los otros grupos. Los resultados de absorbancia mostraron una mayor formación de biofilm para el grupo NbTi, seguido por Acero, NiTi y TiMb ($p<0.05$). Los resultados de EDS confirman las composiciones propuestas por el fabricante. Se concluyó que el tipo de aleación en los arcos ortodónticos tiene un efecto en la rugosidad superficial. Las características químicas y morfológicas de los arcos están relacionadas con la adhesión del biofilm de *S. mutans*.

Descriptores: Aleaciones; Ortodoncia; *Streptococcus mutans*; Alambre.

INTRODUCTION

In orthodontic therapy, several fixed and mobile orthodontic devices are used in order to position the teeth and bone structures correctly, providing the patient with adequate functionality and aesthetics[1-5]. The great challenge found in the use of these devices is that their presence in the oral cavity provides retentive areas, which associated with predisposing factors such as diet, poor sanitation and psychosocial factors, can induce greater retention of dental biofilm. This fact can generate a drop in the pH level, causing the appearance of white spots that would culminate in the beginning of the process of caries disease[6,7]. as well as contributing significantly to gum inflammation[8,9].

Within this context, white spot lesions are a major concern for orthodontists, as these tooth enamel demineralization may be irreversible[10,11]. Studies show that efficient oral mechanical hygiene, dental flossing and the frequent use of oral antiseptics are essential for the control of biofilm in patients during orthodontic treatment[12-16]. Orthodontic archwires are present throughout the orthodontic treatment period contributing significantly to the demineralization of the enamel of teeth, making it difficult to have proper access to hygiene[11,17,18].

Studies have been conducted in the last decades in the biomedical area, where titanium, niobium and zirconium alloys have a great relevance for metallic biomaterials, due to their high biocompatibility[17,18]. Among the metallic particles, Niobium stands out, which has been studied in different areas such as the manufacture of metallic alloys, electronic ceramics and catalysts[19]. Niobium oxide (Nb_2O_5) has been used on the surface of dental implants increasing its osseointegration capacity due to its excellent biocompatibility[20]. This particle has been incorporated into an adhesive, incorporating bioglass and niobium phosphate, which has shown the potential of this particle to inhibit the growth of *Streptococcus mutans*, preventing the formation of white spot lesions[14,15].

Another type of relevant alloy within the wire study is steel, which is widely used in orthodontics. Austenitic type steel, due to the presence of ferro-nickel-chromium alloys in its composition, has become very popular in orthodontics due to its low cost and excellent mechanical properties such as good conformability, biocompatibility, ductility, and resistance to corrosion[21,22]. There are reports in the literature that stainless steel containing copper and niobium, showed a significant decrease in biofilm proliferation and planktonic growth in heat treatment, being indicated for use in hospital appliances and domestic utensils[23].

Among these different particles, NiTi is considered the pioneering landmark of changes in orthodontic materials, awakening significant interest in biomechanical applications. Among its main features, the shape memory effect has an important clinical relevance and is used in the early stages of orthodontic movement. But it also has biocompatibility, and at reduced temperature gives greater elasticity[24]. One of its main disadvantages is the presence of Nickel, patients allergic to this metal and its limited formability[25].

And finally, titanium and molybdenum-based beta-titanium alloys have been widely used in orthodontic practice. It has a lower rate of load deflection when compared to other metals and good conformability. As it does not contain nickel in its composition, this is an option for orthodontic treatment for sensitive patients allergic to this metal[25].

Biomaterials industry has sought to modify the surfaces of these materials, in an attempt to improve their mechanical and chemical properties, aiming to increase biocompatibility, reduce corrosion and thereby promote characteristics that are beneficial to the biological balance of the patient's oral environment[26,27]. Given the above, and the clinical relevance of the types of alloys of choice within orthodontic therapy, the purpose of this study was to evaluate the chemical and morphological characteristics and bacterial adhesion of orthodontic arches composed of different metal alloys. The null hypotheses of this study were: 1) Orthodontic arches composed of different metallic alloys do not present different values of surface roughness; 2) Arches composed of different alloys do not present a difference in bacterial adhesion on their surface.

MATERIAL AND METHOD

- *Experimental Design*

The sample size was calculated in the G Power* program (Dusseldorf, Germany), considering the 5% significance level the effect size greater than 0.48. With this, the segments (n=10) were allocated to the following groups: 1) Tru-Chrome- Rock Mountain steel wire (Colorado-USA); G2) NiTi- Rock Mountain wire (Colorado-USA); G3) Bendaloy TMA Rock Mountain wire (Colorado-USA); 4) NbTi Gummetal- Rock Mountain wire (Colorado- USA). The orthodontic arches were segmented (20mm) and sterilized by means of ultraviolet light. After the growth of biofilm on the arches, qualitative and quantitative analysis of biofilm, Scanning Electron Microscopy/Energy-dispersive Spectroscopy (SEM/EDS) and Surface Roughness by means of Confocal Laser Scanning Microscopy (CLSM) were performed.

- Wire cutting

Each arc was sectioned with a cutter in the distal coated portion of 20 mm of wire length. After the cut, two samples were obtained from each arch. The cut was performed with the aid of an orthodontic cutter.

- *Surface Roughness Analysis*

The Confocal Laser Microscope (LEXT OLS4000®, Olympus, Tokyo, Japan) coupled with the OLS4000 software (Olympus, Japan) was used to analyze the surface roughness of orthodontic archwires. The roughness of the archwire surfaces (n=10) were analyzed at µm, with an area of approximately 0.5 mm^2. Three measurements were performed in different regions in the same specimen, considering the mean as the roughness value of the specimen.

○ *Scanning Electron Microscopy/Energy-Dispersive Spectroscopy*

The morphological analysis of the arc surfaces was performed in a scanning electron microscope (JSM 5600 LV; JEOL, Tokyo, Japan) at a voltage acceleration of 15 kV by a single calibrated operator. Energy-dispersive Spectroscopy (EDS) analysis was also performed identifying the qualitative chemical composition of the arcs. For the EDS analysis, the coating was performed with carbon.

○ *Biofilm Quantitative Analysis of adhered microorganisms*

The strain of *Streptococcus mutans* used for the biofilm assay was UA159 (isolated from children with caries – ATCC). For all experiments, strains were inoculated from frozen stocks (at -70oC) in Petri dishes containing BHI agar and incubated for 24-30 h at 37oC in a 10% CO_2 atmosphere. After this period, some colonies were collected and transferred to test tubes with 5 ml of BHI medium and incubated under the same conditions as above. To analyze the formation of mature biofilm, after 18 hours, assays were performed in 96-well polystyrene plates with flat bottom of the brand (Global Plast). Stems were made from each material and sterilized using UV light Stems were made from each material and sterilized using UV light for 30 min (15min/side) with intensity of 30 W/m^2. They were transferred in quadruplicate to the plate wells where 200µL of UA159 strain culture in exponential phase was inoculated (A550nm 0.3) and diluted 1:10 in BHI supplemented with 1% sucrose. The plates were incubated (37°C) in aerobiosis under agitation (Nova Instrument-Thermo-shaker plate shaker, 240 rpm) for 18 h. After this process, the supernatant was removed and the plates were washed three times with distilled water to remove cells that were weakly adhered, and the biofilms were stained with 200µL of 1% aqueous crystal violet solution for thirty minutes and again washed three times and dried at room temperature for 20 min. Using absolute ethanol in a volume of 200µL, the dye adhered to the biofilms was then solubilized for 30 minutes and then transferred to a new 96-well plate with a volume of 100µL and its absorbance (A575nm) was determined in an ELISA reader (Versa MAX, molecular devices, USA). Absorbance measures were expressed as indirect measures of biofilm biomass. Two independent experiments were carried out.

○ *Biofilm formation on the wires*

In order to analyze the initial formation (4 hours) of *S. mutans* Biofilm under the material, an electronic scanning microscope was used, following the protocol of previous study[29] with some modifications. A sample of each material was placed in a 24-well plate together with 1.5 mL of BHI medium plus 1% sucrose. The media were then inoculated with cultures of the strains in half of the exponential growth phase adjusted to the same absorbance (A550nm of 0.03) and the plates incubated at 37°C for 4 hours in aerobiose by agitation (Nova Instrument-Thermo-shaker plate agitator; agitation at 250 rpm). After incubation, the culture medium was removed with sterile micropipettes, and the laminules were washed with 1.5 mL of 0.9% saline solution (NaCl 0.9%) during 15 min. of stirring (MicroPlates Agitator - MA 562, Marconi Equipamentos, Brazil). The washing procedure was repeated for two more times. Then, the laminules were treated with 800 µL of 2.5% glutaraldehyde solution (Sigma- Aldrich) for 30 min, at room temperature. The specimens were then dehydrated by incubation with ethanol solutions in increasing concentrations from 50% to 100% (15 min. incubation for each solution). After dehydration, the samples dried at room temperature and mounted in stubs, to be metallized with gold and analyzed under the microscope JSM 5600 LV (JEOL, Tokyo, Japan) scanning electron microscope. Representative images of the specimens were obtained under a 1,000X magnification. The experiments were performed in duplicate[29].

○ *Statistical Analysis*

The statistical analysis was performed through the R (R Core Team-2018) program. R. A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria). The data regarding surface roughness and absorbance were evaluated by the normality and heterogeneity test. The ANOVA (one-way) and Tukey's post-test were used for data normality. The significance level of $\alpha=0.05$ was adopted. The analysis of MEV/EDS photomicrographs was obtained through exploratory analysis of the images.

RESULTS

For the surface roughness analysis of the segments of orthodontic arches it was observed that the steel arches had lower mean surface roughness compared to the other groups ($p=0.0221$). The TiMb and NiTi groups presented lower mean values when compared to NbTi, which presented the highest surface roughness value (p=0.0349). Means followed by different letters differ significantly from each other (p<0.05) (Table 1).

Figure 1 shows the surface of the threads with different alloy compositions, which are homogeneous within their composition characteristics. Figure 1 also shows the Dispersive Energy Spectroscopy graphs, where the presence of ions was observed and proven, which were reported in its manufacturing process. The steel arc presented the presence of Fe, Ni, Cr ions, being its predominance in Fe. The other alloys presented a higher presence of Ti accompanied by a lower concentration of the other proposed ions.

Table 1. Mean (Standard Deviation) of the Surface Roughness of orthodontic archwires.

Groups	µm
Steel	0.45 (0.04)a
TiMb	0.85 (0.16)b
NiTi	0.75 (0.08)b
NbTi	1.26 (0.14)c

Significance level = α=0.05

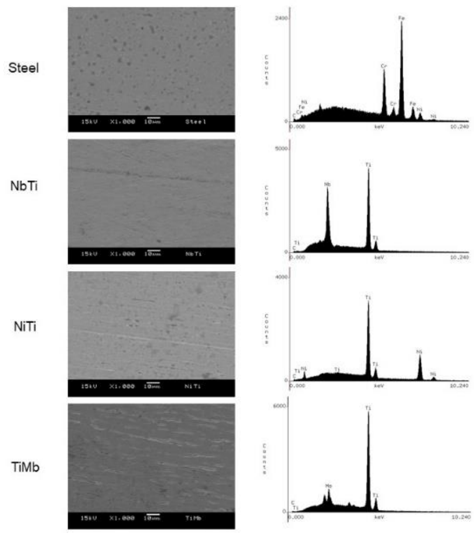

Figure 1: Micrographs and Graphs of Energy Dispersive Spectroscopy of orthodontic archwires by Scanning Electronic Microscopy. Steel, NbTi, NiTi, TiMb

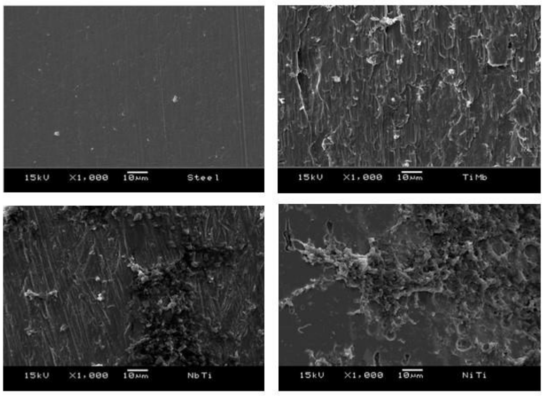

Figure 2: Micrographs of orthodontic archwires after formation of bacterial biofilm by scanning electron microscopy.

Based on Figure 2 it could be observed that steel archwires have lower adhesion of *streptococci mutans* biofilm when compared to the other alloys analyzed. NbTi and NiTi alloys present qualitatively higher adherence and biofilm structure when compared to TiMb arc.

Figure 3 shows the absorbance values, where higher values of cellular metabolism could be observed for the NbTi group, compared to the other experimental groups (p=0.0232). The Steel and NiTi groups presented higher mean absorption values compared to the TiMb group (p=0.0315).

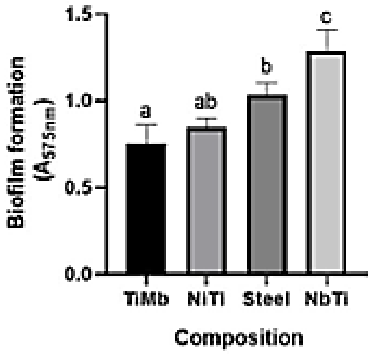

Figure 3: Graph with mean absorbance values of 24h *Streptococcus Mutans* Biofilm Formation. Means followed by different letters differ significantly from each other (p ≤0,05).

DISCUSSION

Currently, through research in the metallurgical field and the advancement of science in orthodontics, several types of metal alloys have been developed, expanding the variety of archwires and orthodontic mechanics. This fact brings new therapeutic approaches and also aims to remedy some difficulties encountered in the use of fixed aparatology, such as friction and the accumulation of biofilm, which can cause the dissolution of adjacent enamel[27].

One of the aspects evaluated in this study was the surface roughness of arches with different compositions. Based on the results, the steel wire presented lower roughness in relation to the group of archwires containing titanium in its composition, thus being the first hypothesis null rejected. Within the groups that contain titanium in its composition, lower roughness values were observed for the NiTi and TiMb groups when compared to NbTi, archwires generate the effectiveness of tooth movement during orthodontic treatment and the greater their roughness, the greater the friction force, reducing by 50% or more the orthodontic force, decreasing the quality of movement and increasing treatment time[28].

The archwires obtain their final shape through the drawing process, which may cause scratch marks on the surface of their alloys, influencing the various types of material properties, such as biological responses[32]. The roughness of the archwires is related to the capacity of resistance to corrosion, the machining process and the quality of the surface finish. Steel archwires have resistance to corrosion, ductility and thermal resistance and their machining process is done by electrical discharge, giving low surface roughness of materials composed of this alloy. Titanium composite archwires, on the other hand, undergo a process of adhesion manufacturing and cold welding, and this fact provides greater roughness to dies or rolls during wire processing[28,29].

Studies report that metal alloys containing copper, silver, have an efficient antimicrobial potential, preventing the adhesion and growth of bacteria, restricting the formation of biofilm[29,30,31]. This modification in the surface of the archwires leads to the prevention of the development of a structured biofilm and it can be a mechanism for the prevention of possible appearance of white spot lesions to the dental tissue during orthodontic treatment[11]. In addition, intraoral exposure of the archwires alters the microstructural aspects and chemical composition of the arches, where acidogenic bacteria alter the capacity of corrosion of the archwire, altering the surface properties of this archwire, making it more irregular and rough[32].

In the present study, the steel archwire had the lowest roughness due to its topography of longitudinal grooves, shallow pores, and small elevations in the surfaces, which can be attributed to the manufacturing process, such as the manufacturing technique and different surface treatments[33,34]. However, when the amount of biofilm was analyzed, it presented intermediate values, and the second hypothesis was rejected. These values can be justified by the free energy of the surface and the physical-chemical properties, that after the washing with PBS of the pioneer planktonic cells, the non-adherent cells went to the medium, where a level of absorbance was measured, when compared to the other tested archwires[33]. The amount of biofilm present for the NbTi arches showed higher values when compared to other groups, once this alloy promotes greater adhesion and bacterial proliferation[34].

Also in this study, different characteristics and compositions of orthodontic arches were evaluated, as demonstrated above in the graph of dispersive energy spectroscopy. It was observed that steel archwires have approximately 8% to 12% nickel, 17% to 12% chromium and varying proportions of manganese, titanium, iron and copper. Due to the presence of chromium, this archwire has resistance to corrosion, also presenting high stiffness, which allows bending, and has a lower coefficient of friction resulting in low friction, being widely used in sliding mechanics for closing spaces and in the final phase of treatment[30].

In this sense, the NiTi archwires presented in this study are composed of 55% Niquel, 45% Titanium and 1.6% cobalt. They have properties such as high flexibility, shape memory and low modulus of elasticity, compared to steel archwires[32]. This low load/deflection property is very favorable in the initial phase of orthodontic treatment, always maintaining a smooth and continuous strength, but it does not present formability[30].

Another group of archwires studied was TMA (Titanium-Molybdenum) which has in its composition 79% Titanium, 11% Molybdenum, 6% Zirconium, 4% Tin, having as characteristics, high formability, high elastic recovery, 50% of the rigidity of the steel archwire, twice its resilience, high coefficient of friction and higher friction[35].

However, an alloy recently studied and developed for biomedical applications, gummetal, composed of Ti-29Nb-13Ta-4.6Zr, does not contain heavy metal (copper), can be used by patients allergic to nickel for not presenting this component in its composition, and its modulus of elasticity is very low (high flexibility). Normally this characteristic in a metallic alloy prevents it from receiving bending, but the gummetal presents high formability. Its rigidity is 20% lower than TMA, 70% lower than SS and 50% lower than NiTi, it has low Young modulus (malleable), which would leave it with low resistance, but instead this alloy has high resistance. It does not present hysteresis, therefore, the applied force takes longer to decrease. With this, the activation remains for longer, maximizing the activations, lower coefficient of friction facilitating the dental movement and accepting electric welding, facilitating the placement of accessories[35]. All these features are very relevant for the application of orthodontic mechanics and especially in Geaw mechanics (Gummetal edgewise archwire), with intrusion movements and verticalization of the posterior region for correction of occlusal inclination through activations that require light and constant force and torque control[35].

CONCLUSION

Within the context of chemical and morphological aspects of orthodontic wires with different metallic alloys and their relationship with biofilm adhesion, it can be concluded that the type of alloy of orthodontic wires influences surface roughness. The chemical and morphological characteristics of the threads are related to adhesion on their surface.

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CONFLICTS OF INTERESTS

The authors declare no conflicts of interests.

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