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INFLUENCE OF ARTIFICIAL SALIVA AND ESSENTIAL OIL-BASED SOLUTIONS ON THE ELECTRICAL PARAMETERS OF NITINOL AND STAINLESS STEEL ARCHWIRES

VPLIV UMETNE SLINE IN RAZTOPIN ETERIČNIH OLJ NA ELEKTRIČNE LASTNOSTI ORTODONTSKIH ŽIČK IZ NITINOLA IN NERJAVNEGA JEKLA

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This paper analyzes the variation in the electrical characteristics of NiTi and stainless steel archwires under exposure to different liquid media and temperatures. The archwires were enclosed in a microfluidic chip to obtain a controlled environment, mimicking oral cavity conditions. Five liquids were selected for testing: artificial saliva, Listerine mouthwash, cinnamon essential oil added to artificial saliva, eucalyptus essential oil added to artificial saliva and orange essential oil added to artificial saliva. Impedance spectroscopy was performed at three temperatures, (25, 37 and 50) °C, and in three different time intervals: at the start of the experiment, after three weeks and after six weeks of exposure. The impedance, its modulus and phase, and Nyquist diagrams were plotted, as well as the root-mean-square (RMS) deviation parameter. The results showed that both materials exhibited comparable responses with small changes over time.

Keywords: NiTi alloy, stainless steel, corrosion, essential oil, impedance spectroscopy

Avtorji opisujejo analizo sprememb električnih lastnosti ortodontskih pritrjevalnih žičk iz zlitine na osnovi Ni in Ti (Nitinol) in nerjavnega jekla pod vplivom različnih raztopin in temperature. Preiskovane žičke so bile zaprte v folijo z mikrofluidom. S tem je bilo doseženo kontrolirano okolje, podobno pogojem v ustni votlini. Za testiranje so uporabili pet različnih raztopin (umetno slino, ustno vodico Listerin, cimetovo, evkaliptusovo in pomarančno eterično olje). Vsa tri eterična olja so bila raztopljena v umetni slini. Meritve z impedančno spektroskopijo so izvajali pri treh različnih temperaturah (25, 37 in 50) °C, in treh različnih časovnih obdobjih izpostavitve. Meritve so opravili na začetku preizkusa, po treh in po šestih tednih. Določili so impedanco, njen modul, fazo, izrisali Nyquistove diagrame in izračunali povprečni kvadratni koren odstopanja. Rezultati analize so pokazali, da imajo vsi preiskovani materiali primerljive lastnosti z majhnimi spremembami glede na čas trajanja preizkusa.

Ključne besede: zlitina NiTi, nerjavno jeklo, korozija, eterična olja, impedančna spektroskopija

1 INTRODUCTION

Metals are ideal for biomedical applications because of their superior mechanical strength and corrosion resistance. Biomedical metallic devices are medical or dental devices that are designed to be used in various parts of the human body. A variety of chemical reactions occur on the surface of a material once it is placed intraorally. At the material-to-human-body-environment-system junction, all biomaterial surfaces form an interface. Biomaterials have two key characteristics: biofunctionality and biocompatibility.[1] Not all metals are suitable for biomedical applications as these two stringent properties must be present. With the advancement in surgical instruments and biomaterials, as well as the rising demand for biomaterials for the oral cavity, the use of biomaterials in the medical field is a growing area of interest.[2]

Nitinol is a name for a group of titanium-based intermetallics with nearly equal amounts of nickel and titanium. Because of the thermoelastic martensitic transformation, nitinol has shape-memory and superelastic properties.[3–5] The shape memory effect and superelasticity in near-equiatomic NiTi alloys are caused by the thermoelastic martensitic transformation from the parent austenite phase with the B2 structure to the monoclinic (M) or rhombohedral (R) martensitic phase.[6–8]

Fabrication of stainless steel is easier than that of Ti-alloys. The microstructures of stainless steel (SS) are austenite, martensite and ferrite. The properties of these structures reflect the fact that they are results of proper chemistry adjustments. The austenitic stainless steel used in orthodontics contains approximately 18 *w*/% Cr and 8 *w*/% Ni. The most important feature of stainless steel is its homogeneous corrosion resistance. On the other hand, stainless steel has a bad reputation regarding its use in body-fluid conditions due to pitting, intergranular, crevice and friction corrosion.[9,10]

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Corrosion of any kind releases a variety of harmful metal ions, such as nickel ions, that have negative effects on the human body.

To resist corrosion, stainless steel and titanium alloys rely on the formation of a passive-surface oxide film. However, even if such a protective oxide film is present on a metal surface, metal ions can still be released.[11] Not only is the protective oxide layer susceptible to a mechanical and chemical breakdown, but it can also dissolve over time. While a metal disintegrates due to the presence of oxygen in the environment, some metals and alloys suffer from corrosion and deterioration due to the acidic environment of the oral cavity, the presence of fluoride ions in toothpastes and mouthwashes, and other biomedical solutions.[12]

Nickel compounds have been classified as human carcinogens by the International Agency for Research on Cancer (IARC).[3] Nickel has been shown to have toxic, carcinogenic and immune-sensitizing properties.[4,5] The release of nickel from medical devices may contribute significantly to an oral nickel intake of up to 1 mg per day.[6–8] Nickel has the potential to cause genotoxicity, neurotoxicity, gastrointestinal, cardiovascular, muscular, dermal, metabolic, immunological and carcinogenic effects.[13]

Acidic foods and beverages, such as soft drinks, are constantly attacking metals in oral appliances, promoting the cathodic corrosion reaction and, as a result, also the anodic corrosion reaction (metal dissolution). The effect of pH on nitinol corrosion has been extensively studied. In 2003, Huang et al. measured the amount of ions released from NiTi wires immersed in artificial saliva with various pH values as a function of time.[14] They discovered that, in all conditions, the amount of metal ions released increased with the immersion time, and that their amount was higher when larger amounts of acidic solutions were used.

Fluoride-containing mouthwashes are recommended for the patients with stainless steel crowns, orthodontic appliances and immobilization appliances. Most of these patients are children and adolescents who do not always maintain good oral hygiene and are at high risk of developing caries. To reduce the prevalence of caries, dentists frequently prescribe fluoride-containing mouthwashes on a daily or weekly basis. Fluoride mouthwashes are typically available in concentrations of 0.05 % and 0.2 % of fluoride ions. Due to its ionic, thermal, microbiological and enzymatic properties, the oral environment aids the biological degradation of a metal. As a result, patients are likely to be exposed to corrosion processes to some extent.[15–17]

Fluoride promotes the formation of calcium fluoride spheres that adhere to the teeth and stimulate remineralization while also protecting against acid attacks. Mouthwashes with fluoride help to prevent cavities and protect tooth enamel. Essential oils, which contain high-concentration terpenic hydrocarbon mixtures, have a lot of potential for naturally treating oral infections. Lavender, eucalyptus, peppermint, clove and cinnamon oils are the most important essential oils for maintaining oral health. The antimicrobial properties of these essential oils have been well documented.

Cinnamon oil (CO), derived from cinnamon plants in the Lauraceae family, has been extensively studied for its antibacterial properties and has been shown to be effective against oral bacteria. Its antibacterial properties against Streptococcus mutans make it ideal for preventing dental caries. Because of their powerful antimicrobial properties, natural mouthwashes have been shown to be effective in treating gingivitis and plaque. Herbal extracts of medicinal plants have been used in dentistry as plaque antimicrobial agents, antioxidants, analgesics and antiviral agents to prevent histamine release due to fewer side effects and low toxicity. The biologically active compound eucalyptol is found in Eucalyptus globulus essential oil, also known as eucalyptus oil, which is used as an endodontic solvent in dentistry for mouthwash and dental preparations.[18–22]

With the advancement of modern wireless technologies and wearable electronic devices, the oral cavity is becoming a place for diagnostics as well as therapy. Smart orthodontic brackets, tooth-mounted sensors and biosensors for detecting various parameters in the oral cavity have been reported recently. However, there is a lack of research data on how to determine the electrical performance of metallic dental appliances, and how they perform within a microfluidic chip.[22–24]

The aim of this work was to investigate electrical properties of the most often used metallic dental materials made from NiTi and SS. These characteristics were studied in different fluidic environments, such as artificial saliva, a commercial essential oil based mouthwash and two experimental essential oils added to artificial saliva. To mimic the oral cavity environment, we designed a microfluidic chip as a system for analyzing the variation in the electrical parameters of the NiTi and SS archwires through their exposure to different fluids and temperatures.

2 EXPERIMENTAL PART

2.1 Archwires and microfluidic chip design

Two as-received commercial archwires (from Astar Orthodontic Inc., China) with dimensions of (0.4826 × 0.635) mm were used in this study (**Figure 1a**). The wires were randomly chosen from a packet containing ten archwires. Unlike some other studies, where a cut from the end of an archwire was used, we performed an experiment on the whole archwire, with the intention to imitate the real application of a complete archwire, being a part of an orthodontic appliance in the oral cavity.

For the fabrication of the microfluidic chips, 120-μm-thick PVC foils were used in this study. Each

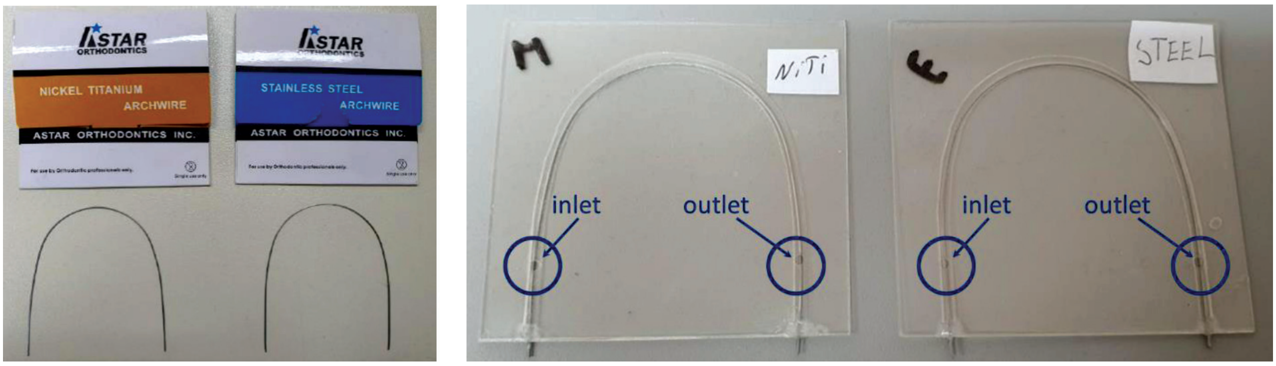

Figure 1: Archwires: a) NiTi and SS wires, b) wire integration into a PVC microfluidic chip

chip was made of five layers of foil. The drawing of individual layers of a microfluidic chip was performed in the AutoCad program, **Figure 2**. The first layer is full foil, the last layer has two small round holes for the inlets, through which the solutions are injected into the chip, and the three middle layers are cut in the shape of a wire so that the solution can easily coat the entire wire during the injection. **Figure 1b** depicts the fabricated microfluidic chips with a NiTi wire (left) and an SS wire (right).

A Graphtec CE6000-60 Cutter Plotter was used for cutting the foil. Before the cutting, the setting was made in the Graphtec Pro Studio device software. After loading the design made in the AutoCad program, it was necessary to adjust the position of the cutter plotter knife. When the knife was positioned, the speed and force of cutting the foil were determined. The force and speed had to be carefully determined to avoid damaging the foil (high force and speed) or the skipping of the knife, not cutting all the parts of the foil properly (low force and speed). The final step was to stack the layers of foil onto each other and insert a certain wire into the middle layer of the chip (the microfluidic channel). After everything was put together, lamination was done. An MBL laminator was set to speed 3 and a temperature of 160 °C. The ends of each archwire were protruded from the microfluidic chip by approximately 3 mm, enabling the connections with alligator clips for measuring electrical characteristics.

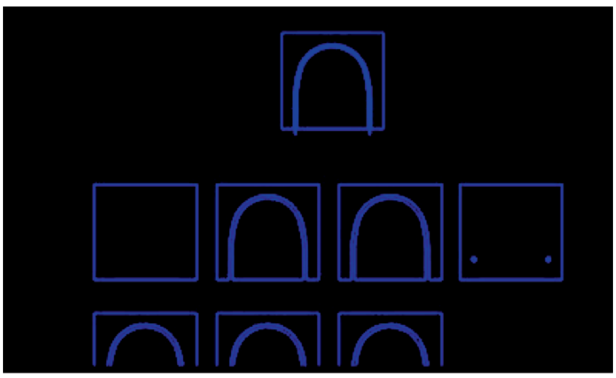

Figure 2: Layer design of a microfluidic chip

2.2 Preparation of the liquids and injections into the chips

Five types of liquids were used in the study:

1) artificial saliva,
2) Listerine mouthwash,
3) cinnamon essential oil added to artificial saliva (dilution 1 : 100),
4) eucalyptus essential oil added to artificial saliva (dilution 1 : 100),
5) orange essential oil added to artificial saliva (dilution 1 : 100).

Narrow pipette-shaped syringe tips were used to inject the above liquids into the inlets of the microfluidic chips.

2.3 Instruments

The devices used for the testing included:

1) PalmSens4 galvanostat/potentiostat,
2) PC with installed PSTrace 5.8 software,
3) adjustable hot-air source,
4) thermal infrared camera (ULIRVision, China).

Electrochemical impedance spectroscopy was applied using the PalmSens4 instrument. To start the measurement, the PalmSens4 device was connected to the PC with the PSTrace software via Bluetooth. The first mea-

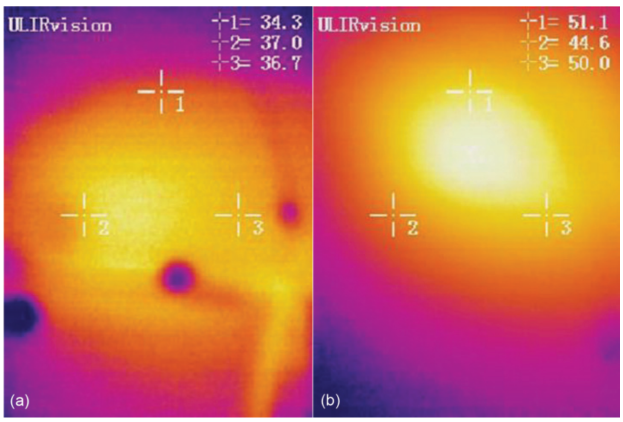

Figure 3: Thermal-camera view of the archwires heated to: a) 37 °C and b) 50 °C

surement was performed on a sample of the chip with the NiTi wire and sample of the chip with the SS wire without injecting the solution. We performed three consecutive measurements for each chip at different temperatures, at room temperature (25 °C), 37 °C and 50 °C. We used the hot-air source to change the temperature in the chip, and we monitored the exact temperature with the thermal camera that can be seen in **Figure 3**. All the measurement data was stored as excel files and plotted via the GraphPad Prism software.

3 RESULTS

The main purpose of the study was to evaluate the influence of the mouthwash and different essential oils on the archwires made of two different materials. In order to achieve the main goal, the impedance of the archwires enclosed in the microfluidic chips was obtained. An EIS analysis was chosen over a physical-optical cross-examination as it provides several important advantages:

1) EIS is non-invasive and it does not require any specific pre-treatment of the sample;
2) EIS can be performed in-situ with low-cost readout devices, reducing the overall costs and increasing accessibility to real-life in-vivo applications;
3) EIS provides the response of a complete sample, rather than just one small part which is very common in the case of a physical-optical cross-examination.

The impedance of the NiTi and SS archwires was measured firstly in a microfluidic chip without any liquid inside. These measurements are marked as Empty in the subsequent graphs. Next, the liquids were injected into the chips and measurements were made and repeated after 3 weeks and 6 weeks. The impedance as the modulus of all samples is depicted in **Figure 4**. The graphs indicate the types of archwire (SS – left, or NiTi – right) and the temperatures: 25 °C – first row, 37 °C – second row and 50 °C – third row. Moreover, the liquids are marked with colors according to the legend below the graphs (the same for each graph) and the time is marked as follows: a full line for the first measurement, a dashed line for the measurements after 21 d (3 weeks) and a dotted line for the measurements after 42 d (6 weeks). The impedance phase of all the samples following the same pattern and labelling is presented in **Figure 5**. In these figures the only clear difference in the impedance is noted between the empty chips and filled ones.

The differences in the impedance caused by the application of selected liquids and the time are not clearly visible. Because of that, Nyquist plots were also included in **Figure 6**, with the same notation and color scheme as used for the impedance modulus and phase angle. The Nyquist plots present the real part of impedance (Z') versus the inverse-signing imaginary part of impedance (Z''). If a Nyquist plot shows a semi-circle capacitive loop, its diameter is directly proportional to the corrosion

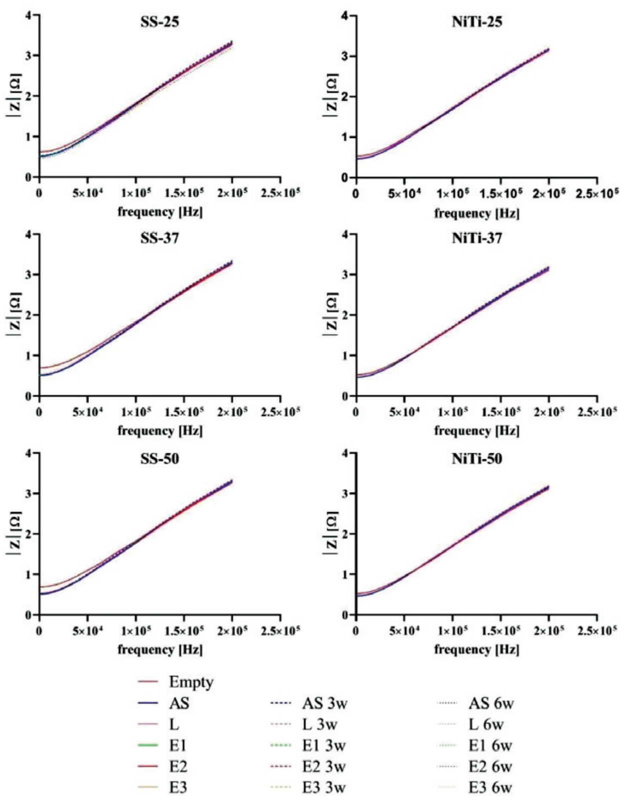

Figure 4: Modulus of the impedance for all studied samples

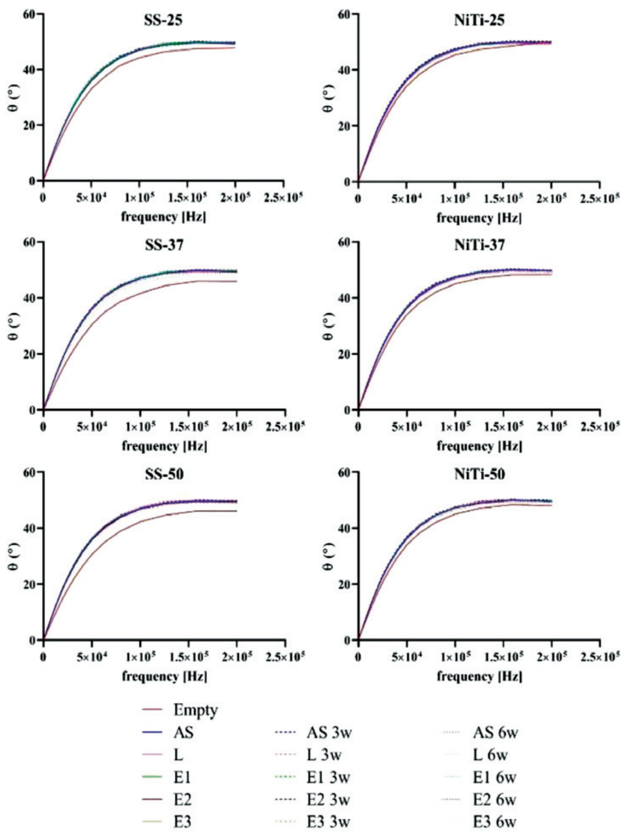

Figure 5: Measured phase angle for all studied samples

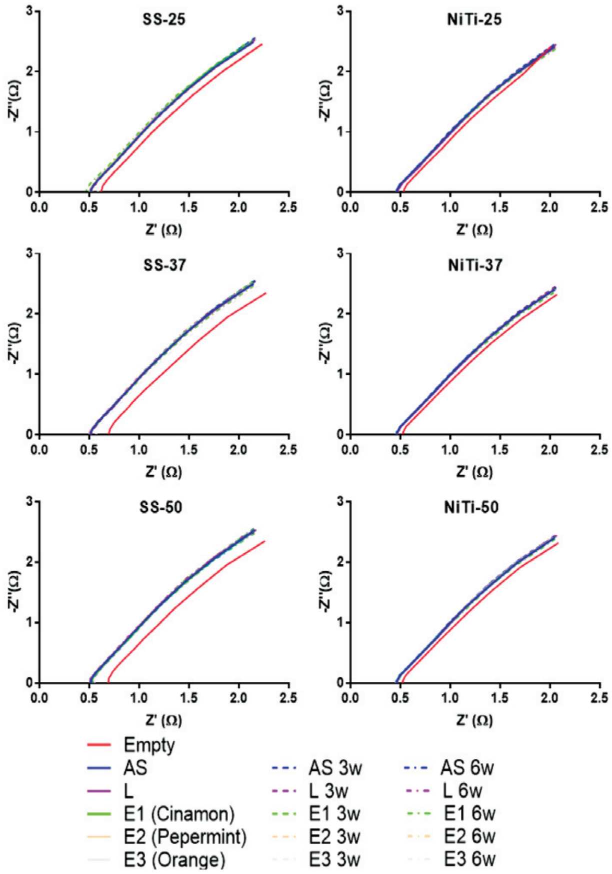

Figure 6: Nyquist plots for all analyzed samples

resistance of the sample for the corroding system under kinetic control. Thus, by comparing the diameters without fitting, one can compare the behaviors of two samples in the same medium, or the effects of any tested parameters on the behavior of the system. However, the differences between the Nyquist plots caused by different liquids and times are not clearly visible. Because of that, no equivalent circuit modelling was performed as it was reasonable to expect that very similar results would be obtained for all 108 configurations, not providing new useful data. As it can be seen from **Figure 6**, the plot of Z'/Z'' for various solutions varies more with the increasing temperature for SS. This can be attributed to the higher temperature resistivity of SS, when compared to NiTi, causing higher temperature resistivity of the SS wire without any solution (red curves in **Figure 6**).[26,27] Despite the fact that a visual observation of impedance spectra or Nyquist diagrams can provide initial clues regarding the corrosion level, more precise metrics are needed to quantify the level for different materials exposed to corrosion. One such parameter is the root-mean-square deviation (RMSD) of the real part of impedance.[28] The RMSD for the real part of impedance is defined with Equation (1):

$$RMSD = \sum_{i=1}^{N} \sqrt{\frac{\left((\mathrm{Re}(Y_{i,1}) - (\mathrm{Re}(Y_{i,2}))\right)^2}{\left(\mathrm{Re}(Y_{i,1})\right)^2}} \quad (1)$$

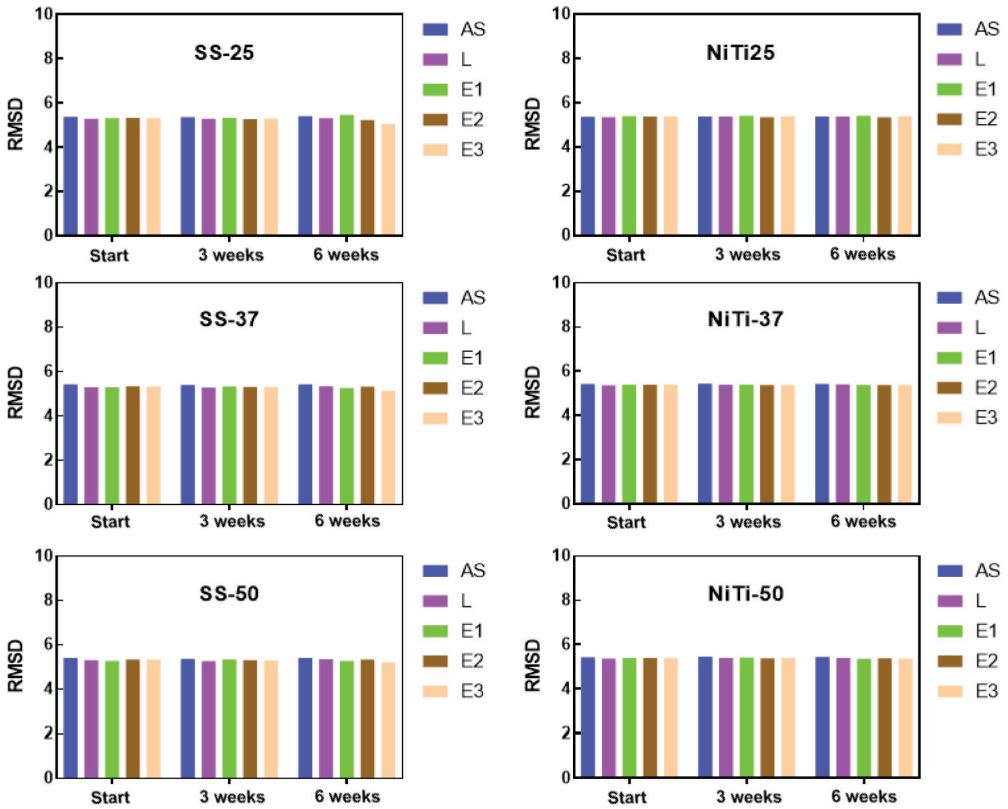

Figure 7: RMSD values for all measured samples

where the real part of impedance of the first measurement (no solution) is $\mathrm{Re}(Y_{i,1})$, while the real part of impedance for different solutions is $\mathrm{Re}(Y_{i,2})$ and N is the number of measurement points in a frequency sweep. The result is a scalar value, which can easily be used to compare corrosion levels of different materials when compared to the baseline (empty). The RMSD values were calculated and presented in **Figure 7**, with the aim to clarify the influence of selected liquids on the archwire corrosion.

As can be seen, both materials in all five solutions demonstrated very similar responses, with small changes over time (from the initial measurement to six weeks afterwards).

4 DISCUSSION

The oral cavity is one of the most hostile environments in the human body, with temperature and pH variations that are greater than in most other parts of the body. The corrosion caused by the graded degradation of materials due to an electrochemical attack is a cause for concern, especially when materials are exposed to the aggressive electrolytic environment of the human mouth. Corrosion is unavoidable and its consequences can jeopardize the material mechanical and biocompatibility properties.[29] Immersion tests revealed that at a pH of 4.2, the highest release of Ni ions occurred, while the surface morphology of orthodontic appliances changed as they became inhomogeneous and rough.[27]

Various synthetic physiological fluids are used to simulate bodily, oral, dentinal or bony conditions. The most reliable and similar medium for testing the properties of biometals used in dentistry is artificial saliva. With this in mind, during this experiment, artificial saliva was used as a solution for analysis, but also as a solution to which three experimental essential oils were added, whose corrosion potential was also tested.

Mouthwashes are becoming increasingly popular as a means of preventing tooth decay, periodontal disease, odors, and for implant maintenance, even for children with metal restorations or metallic appliances. The role and impact of prophylactic agents on corrosion, mechanical properties, surface characterization and friction caused by the sliding motion have all been studied. Mouthwashes, commonly used in orthodontic appliances, have the potential to cause ion leaching from these alloys. Furthermore, metals such as chromium and nickel can produce atom species from molecular oxygen, resulting in superoxide, a highly toxic gas. A nitinol wire behaves passively in a simulated saliva solution, whereas an SS wire exhibits pitting corrosion. Fluoride ions raise the passivity current density in both wires, lowering nitinol breakdown potential while inverting the effect on SS.[28]

Application of green inhibitors based on plant extracts and essential oils (EOs) are very important in dental medicine. EOs have antibacterial, antifungal effects and can be used against biofilm forming on Ti and SS surfaces.[30] The effects of NaF and the extracts of different plants (Artemisia, clove and Celtis australis) on the corrosion resistance of NiTi and CuNiTi archwires were investigated by Fatene at al.[31] Peppermint essential oil was used as the coating for Ti alloys or SS and mechanical/chemical attributes were determined in the paper.[32] The clove EO and ultraviolet light (UV-C) were combined to inactivate the biofilms on SS.[33]

Several studies used electrochemical impedance spectroscopy (EIS) to investigate the behavior of passive Ti films and some of their alloys in simulated physiological solutions. In most cases, impedance data was analyzed using equivalent circuit models, but it was only found in a few of these studies.

The importance of controlling the temperature during the experiments was already emphasized in the study conducted by Friedli et al. In their investigation, orthodontic wires were stored at four different temperatures (5, 22, 36 and 60) °C for twenty-four hours before being mechanically tested.[34] The obtained results are in agreement with our current investigation. In our study, all the samples showed a uniform low corrosion potential at all testing temperatures and throughout the duration of the experiment.

The Listerine contains four essential oils that are antibacterial antiplaque agents, shown to penetrate biofilms. The oils included 0.092 % of eucalyptol, 0.064 % of thymol, 0.060 % of methyl salicylate and 0.042 % of menthol. In the conducted research, we tested another three essential oils at much higher concentrations, at a dilution of 1 : 100. The following oils were tested: eucalyptol, cinnamon oil and orange oil. With regard to eucalyptus oil, its antimicrobial activity was discovered to be linked to the synergistic effects of the major and minor components rather than the concentration of a single component.[16] It was shown that eucalyptus has antimicrobial activity against both gram-negative and gram-positive bacteria. Trans-cinnamaldehyde, eugenol and linalool are three of the most important components of cinnamomum zeylanicum cinnamon oil, accounting for 82.5 % of the total composition. Cinnamaldehyde is the most active component of cinnamon and it is the main constituent. The antimicrobial effect is based on the growth inhibitory effect on a variety of bacteria, including gram-positive, gram-negative and fungal isolates.[35] The essential oils found in orange peels have a lot of promise as an antimicrobial agent against oral pathogens.[36,37] All the tested samples, a commercial one, Listerine and three experimental ones, used in this study showed very similar corrosion potentials, which did not exceed the corrosion potential of artificial saliva.

In this study, we considered a time period of 6 weeks, with one injection per sample, to provide information on the static response of archwires, which is not comprehensively covered in the literature. The purpose of our study was to validate the assumption that three types of essential oils do not make a strong corrosion impact on any of the two types of wire, and that they are safe to use over a prolonged period of time in various environmental conditions (at three temperatures that were studied).

5 CONCLUSIONS

For the majority of orthodontic patients, using fixed orthodontic appliances is part of the regular treatment. The usual materials applied as archwires are nitinol and stainless steel and their properties were analyzed in this study by exposing them to different solutions containing essential oils, and different temperatures, imitating the conditions in the oral cavity. The modulus of impedance as a function of frequency was measured and a range of 0.6–3.5 Ω was obtained; the SS archwire demonstrated a slightly higher electrical resistance than the NiTi archwire. At low frequencies both archwires had a phase angle of around zero, indicating that they had a resistive character, whereas at high frequencies, the phase angle increased up to 50°, indicating an inductive character. The studied archwires did not demonstrate changes in the impedance as a function of temperature variation. Our findings can be potentially very useful when recommending the use of chemoprophylactic agents to patients with orthodontic appliances. In future, it will be important to analyze the variation in the electrical parameters of these archwires for a period longer than six weeks (presented in this paper) to be closer to real applications where fixed orthodontic appliances are used for up to six months, and to evaluate all the effects of treatment.

Acknowledgment

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