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Korozija nikal-titanijevih ortodontskih žičanih lukova u slini i oralnom probiotskom preparatu

Corrosion of Nickel-Titanium Orthodontic Archwires in Saliva and Oral Probiotic Supplements

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Sažetak

Cilj: Ispitati kako uporaba probiotskih preparata utječe na korozijsku stabilnost ortodontskih žičanih lukova nikal-titanijeve legure (NiTi). **Materijal i metode:** Testirani su NiTi lukovi dimenzija 0,508 × 0,508 mm i duljine 2,5 cm, te (sastav Ni = 50,4 %; Ti = 49,6 %) neobložene, nitrirane i rodirane površine. Površinska mikrogeometrija istraživana je skenirajućim elektronskim mikroskopom pri povećanju od 1000 puta, a površinska hrapavost mjerena je profilometrom te izražena u sljedećim vrijednostima: prosječna hrapavost, maksimalna visina i maksimalna dubina hrapavosti. Vrsta i dinamika korozije ispitani su elektrokemijskom metodom cikličke polarizacije. **Rezultati:** Legura rodirane površine u slini ima značajno veću opću koroziju od nitirirane i neobložene, uz veliku snagu efekta ($p = 0,027$; $\eta2 = 0,700$). Probiotski preparat povećava opću i lokaliziranu koroziju rodirane žice i malo smanjuje opću, te povećava lokaliziranu koroziju neobložene žice, a u slučaju nitrirane žice vrlo je mala mogućnost od pojave korozije. Razlike u površinskoj hrapavosti između nikal-titanijevih žica prije korozije nisu značajne. Izloženost umjetnoj slini smanjuje prosječnu hrapavost rodiranih žica ($p = 0,015$; $\eta2 = 0,501$). Medij ne utječe znatno na površinsku mikrogeometriju nitriranih i neobloženih žica. **Zaključak:** Probiotski preparat utječe na opću i lokaliziranu koroziju, ovisno o vrsti nikal-titanijeve obloge. Povećava opću koroziju rodirane žice te uzrokuje pojavu lokalizirane korozije neobložene i rodirane žice. Probiotski preparat nema značajan utjecaj na površinsku hrapavost, osim utjecaja same sline.

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Ključne riječi
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Uvod

Usna šupljina složena je sredina u kojoj su zubi i okolna tkiva stalno pod utjecajem raznih sila pri gutanju i žvakanju te djelovanjem sline i ostataka hrane. Sastav i pH sline razlikuje se od osobe do osobe. Slina sadržava mješavinu anorganskih soli (uglavnom kloride i fosfate), organske kiseline, enzime, bakterije i želučane izlučevine (1). Kako je riječ o vrlo agresivnoj sredini, bitna je značajka dentalnih materijala biokompatibilnost s okolnim tkivima i organizmom u cjelini, te se očekuje da su postojani na mehanička naprezanja i otporni na degradaciju uzrokovanu djelovanjem sila i korozivne sredine. Pojava korozije u usnoj šupljini može uzrokovati pigmentaciju zuba, a u nekim slučajevima čak i alergijsku reakciju kao posljedicu otpuštanja metalnih iona u organizam (2 – 4).

Osim navedenih posljedica, korozija metalnih dijelova ortodontskih naprava može znatno utjecati i na biomehanička svojstva, pa samim time i na učinkovitost naprava (5). Posljednjih godina ortodonskim se pacijentima, uz to da po-

Introduction

The oral cavity is a complex environment in which teeth and surrounding tissues are under constant influence of various forces caused by swallowing and chewing. Saliva composition and pH differ from person to person. Saliva contains a mixture of inorganic salts (mainly chlorides and phosphates), organic acids, enzymes, bacteria and gastric secretions (1). Since the environment is very aggressive, an essential property of dental materials is their biocompatibility with surrounding tissues and the entire body. In consequence, they are expected to be resistant to mechanical stress and degradation caused by forces and the corrosive environment. Corrosion in the oral cavity causes tooth discoloration, and in some cases allergic reactions as a consequence of metal ions being released into the organism (2-4).

Apart from the abovementioned consequences, corrosion of metal parts of orthodontic appliances can affect their biomechanical properties, whereby it can affect appliance effiboljšaju oralnu higijenu, preporučuje i korištenje probiotskih preparata kako bi se smanjila pojava karijesa i gingivitisa kao najčešćih nuspojava tijekom dugotrajne ortodontske terapije. Probiotici su mikroorganizmi koji povoljno djeluju na ljudsko zdravlje (6). Primjena probiotskih bakterija u usnoj šupljini pokazala je dobre rezultate u terapiji kandidijaze, halitoze, parodontitisa, gingivitisa i karijesa (7 – 11). Zbog oblika ortodontske naprave mogućnost pojave navedenih stanja znatno je povećana kod ortodontskih pacijenata u odnosu na ostatak populacije. Ortodontske naprave otežavaju održavanje adekvatne oralne higijene, čime se oko nje stvaraju uvjeti za razvoj i sazrijevanje biofilma te razmnožavanje patogenih mikroorganizama. Zbog toga je ortodontskim pacijenatima indicirana primjena oralnih probiotskih preparata. Istraživanja utjecaja *Lactobacillus paracasei* i *Bifidobacterium animalis subsp. lactis* pokazala su kod ortodontskih pacijenata značajno smanjenje broja *S. mutans* (12 – 14).

Najčešće probiotske bakterije koje se preporučuju za oralno zdravlje su laktobacili (15). No nema relevantnih istraživanja koja bi pokazala kako probiotski preparati utječu na korozijsku stabilnost ortodonskih žica. Zna se da različite bakterije i gljivice mogu potaknuti koroziju metala izravno svojim metaboličkim djelovanjem, ili stvarajući okružje povoljno za početak korozijskog procesa. Zato je svrha ovoga rada bila ispitati kako uporaba probiotskih preparata utječe na korozijsku stabilnost ortodontskih žičanih lukova nikal-titanijeve legure (NiTi). Hipoteze su bile da, uz korozivno djelovanje sline na ortodontske žičane lukove, korištenje probiotskih preparata tijekom ortodontske terapije može dodatno potaknuti opću i lokaliziranu koroziju. Očekivalo se i da će se utjecaj očitovati u većoj površinskoj hrapavosti žica izloženih djelovanju probiotskog preparata. Pretpostavlja se da će vrsta obloge žice mijenjati sklonost prema koroziji te da će nitriranje površine smanjivati, a rodiranje povećavati tu sklonost.

Materijal i metode

Testirani su žičani lukovi NiTi legure dimenzija 0,508 × 0,508 mm (0,020 × 0,020 inča) i duljine 2,5 cm (sastav Ni = 50,4 %; Ti = 49,6 %) neobložene površine (BioForce Sentalloy®), te nitrirane (IonGuard®) i rodirane površine (High Aesthetic®) (Dentsply GAC, Bohemia, SAD). Ispitano je ukupno 45 žičanih lukova, te je njih 15 od svake vrste žice podijeljeno u dvije eksperimentalne skupine i jednu kontrolnu. Prva eksperimentalna skupina sastojala se od pet uzoraka svih vrsta žica i bila je izložena djelovanju umjetne sline, a druga, u kojoj je bilo također pet uzoraka navedenih žica, bila je izvrgnuta djelovanju umjetne sline uz dodatak probiotika. Neizložene žice (pet uzoraka od svake vrste) služile su kao apsolutna kontrola. Za izračun veličine uzorka uzeti su podaci iz pilot-studije. Uz pretpostavku da će između dva eksperimentalna uvjeta biti razlika u parametru prosječne hrapavosti od 0,021 te standardne devijacije od 0,005 u jednom i 0,013 u drugom uvjetu, dobiva se potrebna veličina od po pet uzora-

ciency (5). Lately, it has been recommended that, in addition to improved oral hygiene, orthodontic patients should use probiotic supplements to decrease the incidence of caries and gingivitis as the most frequent side effects of long-term orthodontic therapy. Probiotics are microorganisms that have a beneficial effect on human health (6). The use of probiotic bacteria in the oral cavity has shown good results in treatment of candidiasis, halitosis, periodontitis, gingivitis and cavities (7-11). Orthodontic appliances are designed in such a way that the incidence of the abovementioned conditions is significantly increased in orthodontic patients when compared to general population. Orthodontic appliances make maintenance of adequate oral hygiene more difficult, whereby conditions are created for the development and maturation of biofilm and for propagation of pathogenic microorganisms around the appliance. Research of influence of Lactobacillus paracasei and Bifidobacterium animalis subsp. lactis has shown a significant reduction in the number of S. mutans in orthodontic patients (12-14).

Most common probiotic bacteria used for oral health are lactobacilli (15). However, there is no relevant research to indicate how probiotic supplements affect corrosion stability of orthodontic archwires. It is known that different bacteria and fungi can cause metal corrosion, either directly through their metabolic influence, or by creating locally corrosive conditions. For this reason, the objective of this paper was to examine how the use of probiotic supplements affects corrosion stability of orthodontic archwires made of nickel-titanium alloy (NiTi). It has been hypothesised that, in addition to the corrosive effect of saliva on orthodontic archwires, the use of probiotic supplements during orthodontic therapy can further propagate corrosion, either generally or locally. It has been expected that the effect would manifest itself in a higher surface roughness of archwires that have been exposed to the effect of probiotic supplement. Also, it was expected that the type of coating would change susceptibility to corrosion and that nitrification would decrease, while rhodium coating would increase susceptibility to corrosion.

Materials and Methods

Testing was performed on NiTi archwires having dimensions 0.58x0.508 mm (0.020x0.020 inch), and the length was 2.5 cm (composition Ni=50.4%; Ti=49.6%), uncoated surface (BioForce Sentalloy®), nitrified surface (IonGuard®) and rhodium coated (High Aesthetic®) (Dentsply GAC, Bohemia, USA). A total of 45 wires, 15 of each type were used. They were distributed in two experimental groups and one control unexposed group. First experimental group consisted of five samples of each type of wire which were exposed to artificial saliva while the second group, also consisting of five sample of each type of wire, was exposed to artificial saliva with addition of probiotic supplement. Unexposed archwires (five samples of each archwire type) served as absolute controls. Data from the pilot study were used to calculate the size of the sample Assuming a difference in the roughness average parameter of 0.021 between two experimental conditions and taking into account a standard deviation of ka po skupini. Kalkulacija je rađena u statističko uz snagu 80 % i značajnost α = 0,05, dobiva se potrem softveru MedCalc 14,8,1 (MedCalc Software bvba, Ostende, Belgija).

Umjetna slina imala je sljedeći sastav: 1,5 g/l KCl, 1,5 g/l $NaHCO_3$, 0,5 g/l $NaH_2PO_4xH_2O$, 0,5 g/l KSCN, 0,9 g/l mliječne kiseline i pH 4,8 (16). pH od 4,8 izmjeren je u jednodnevnom i dvodnevnom plaku i služio je kao simulacija pacijenta s lošom oralnom higijenom (17). Druga eksperimentalna skupina bila je izložena djelovanju umjetne sline istog sastava i pH-a, s dodatkom preparata s probiotskim bakterijama *Lactobacilus reuteri Prodentis* DSM 17938 i ATCC PTA 5289 (BioGaia, BioGaia AB, Švedska). Svaki uzorak žice bio je uronjen u 1 mL eksperimentalne otopine (čista umjetna slina ili umjetna slina s otopljenim probiotskim preparatom u omjeru 1 tableta na 30 mL) u plastičnim Eppendorfovim epruvetama od 1,5 mL (Sigma-Aldrich, St. Louis, SAD). Imerzija ili potapanje trajalo je ukupno 28 dana, a otopine su mijenjane jedanput na tjedan. Radi simulacije temperaturnih varijacija u usnoj šupljini tijekom konzumacije toplih i hladnih napitaka, provedeno je termocikliranje od 2500 ciklusa od 5 °C do 50 °C prvih pet dana na uređaju Thermo Haake Willytech (SD Mechatronik Feldkirchen-Westerham, Njemačka). Širok raspon u broju ciklusa i temperaturi termocikliranja prije toga pronađen je i proučen u literaturi, te se koristi radi simulacije dulje izloženosti materijala ekstremnim temperaturnim razlikama (18). Epruvete s uzorcima i eksperimentalnim otopinama bile su naizmjence uranjane u termalne kupke po 30 sekunda s dvije sekunde na sobnoj temperaturi između uranjanja. Nakon toga su uzorci u epruvetama do kraja eksperimenta bili pohranjeni u inkubatoru na temperaturi od 37 °C. Neizložene žice služile su kao apsolutna kontrola pri usporedbi mehaničkih svojstava. Da bi se minimalizirao utjecaj supstancija dodanih u tabletu nakon otapanja BioGaie u umjetnoj slini, otopina je profiltrirana, a prisutnost probiotskih bakterija u filtratu provjerena je nasađivanjem na MRS agar (Sigma-Aldrich, St. Louis, SAD).

Oblik i dubina nastalih korozijskih oštećenja na površini ovih žica prije izlaganja medijima i nakon toga, ustanovljeni su skenirajućim elektronskim mikroskopom (SEM FEI Quanta-200, FEI Company, Hillsboro, SAD) uz povećanje od 1000 puta sa sekundarnim elektroničkim slikama. Mjerenje površinske hrapavosti obavljeno je kontaktnim profilometrom Talysurf CLI 1000 (Taylor Hobson Ltd., Leicester, Velika Britanija). Ispitivani profili površine mjereni su dijamantnom iglom promjera 5 µm. Tijekom mjerenja igla se kretala stalnom brzinom preko uzoraka i to snagom od 1,3 mN. Mjereno je po pet uzoraka od svake vrste žice i na svakom uzorku su tri varijable: srednje aritmetičko odstupanje profila (Ra), maksimalna visina (Rz) i maksimalna dubina hrapavosti (Rmax) mjerene na tri profila korištenjem Gaussova filtra s graničnom vrijednošću (referentnom duljinom) od 0, mm i duljinom uzorka za ispitivanje površine od 4 mm. Za statističku analizu uzeta je aritmetička sredina dvaju ponovljenih mjerenja. Za kontrolu preciznosti na svakoj su žici provedena po dva mjerenja i graf svakoga očitan je dva puta.

Budući da se korozija metala u usnoj šupljini događa prema elektrokemijskom mehanizmu, korozija žica ispitana je

0.005 for one condition and 0.013 for the other, with power of 80% and significance of α=0.05, a size of five samples per group was obtained. The calculation was done by using statistical software MedCalc 14.8.1 (MedCalc Software bvba, Ostend, Belgium).

Artificial saliva had the following composition: 1.5 g/L KCl, 1.5 g/L NaHCO3, 0,5 g/L NaH2PO4xH2O, 0,5 g/L KSCN, 0,9 g/L of lactic acid, pH 4.8 (16). A pH of 4.8 was measured in one and two days old plaque, which served as a simulation of a patient with very poor oral hygiene (17). Oral probiotic supplement containing bacteria Lactobacillus reuteri Prodentis DSM 17938 and ATCC PTA 5289 (BioGaia, BioGaia AB, Sweden) was added to artificial saliva of the second experimental group Each archwire sample was immersed into 1 mL of experimental solution (pure artificial saliva or artificial saliva containing dissolved probiotic supplement at the ratio of 1 lozenge to 1 mL of saliva) in plastic 1.5 mL Eppendorf test-tubes (Sigma-Aldrich, St. Louis, USA). The immersion lasted for 28 days, and solutions were changed once a week. During the first five days of testing and in order to simulate temperature variations in the oral cavity when consuming hot and cold beverages, thermocycling was performed in 2500 cycles at temperatures from 5oC to 50oC by using the Thermo Haake Willytech (SD Mechatronik Feldkirchen-Westerham, Germany) machine. A wide range of cycles and temperatures for thermocycling have been reported in the literature. Also, it has been stated that the setting simulated longer exposition of the material to the temperature extremes (18). Test-tubes containing samples and experimental solution, one after another, were immersed in thermal baths for 30 seconds, with two seconds at air temperature between immersions. After that, the test-tubes with samples were stored in an incubator where they were kept at a temperature of 37oC until the end of the experiment. Unexposed archwires served as absolute control for comparison of mechanical properties. To minimise the effect of substances added to the tablet after dissolution of BioGaia in artificial saliva, the solution was filtered and the presence of probiotic bacteria in the filtrate was tested by inoculating them onto MRS agar (Sigma-Aldrich, St. Louis, USA).

The shape and depth of corrosive damage on the surface of the archwires before and after exposure to the media were determined by using a scanning electron microscope (SEM FEI Quanta-200, FEI Company, Hillsboro, USA) at 1000x magnification with the secondary electron imaging. Measurement of surface roughness was performed using the contact profilometer Talysurf CLI 1000 (Taylor Hobson Ltd., Leicester, UK). Traced profiles of the real surface were acquired with a diamond stylus of 5 µm radius. During the measurement, the stylus was moved at a constant speed across the samples with a measuring force of 1.3 mN. Five specimens from each wire type were measured, and on each sample, three variables: roughness average (Ra), maximum height (Rz) and maximum roughness depth (Rmax) were measured on three profiles, using a Gaussian filter with a cutoff value of 0.8 mm and the evaluation length of 4 mm. The arithmetic mean of two repeated measurements was used for statistical analysis. For the sake of precision control, two measurements elektrokemijskom metodom cikličke polarizacije na potenciostatu PAR263A i analizatoru frekvencija PAR FRD 1025 (Princeton Applied Research, Oak Ridge, SAD) (19). Mjerenja su obavljena na 300 ml umjetne sline, uz dodatak šest tableta probiotika na temperaturi od 37 ± 2 °C. Ispitivane žice bile su izrezane i izolirane lakom tako da im je izložena površina bila 0,61 cm^2.

Iz polarizacijskih krivulja ovisnosti potencijala o logaritamskoj vrijednosti gustoće struje korozije određena je gustoća korozijske struje (corrosion current density – Icor) koja je ekvivalent brzini opće korozije. Tendencija pojave lokalnih korozijskih oštećenja na materijalu određena je na temelju izgleda polarizacijske krivulje, tj. odnosa između korozijskog potencijala (corrosion potential – Ecor), potencijala pri kojem je zabilježena najmanja gustoća struje, potencijala pucanja pasivnoga oksidnog filma (brakedown potential – Ebd), potencijala pri kojem se naglo povećava gustoća struje te potencijala repasivacije (repasivation potential –Erp), potencijala pri kojem se, u povratnom dijelu krivulje, izjednačava struja s onima iz početnog dijela ciklusa. Pri potencijalu Ebd nastaje oštećenje zaštitnoga pasivnog filma, odnosno legura se intenzivno otapa, što može rezultirati značajnim otpuštanjem iona nikla. Što je veća razlika između vrijednosti Ecora i Ebda, to je manja vjerojatnost da će u realnim uvjetima primjene nastati značajna oštećenja pasivnog filma. Oštećeni pasivni sloj može se obnoviti (repasivirati) u slučaju nižih potencijala u povratnom dijelu polarizacijske krivulje. Ako repasivacija ne nastane pri potencijalima bliskima Ebdu, tada je zanemariva vjerojatnost za pojavu lokalizirane korozije. Nakon završetka elektrokemijskih mjerenja površina ispitivanih uzoraka snimljena je skenirajućim elektronskim mikroskopom sa sekundarnim elektroničkim slikama (Tescan Vega 3, Tescan, Brno, Češka) uz povećanja od 1000 puta.

U statističkoj analizi korišten je t-test te analiza varijance studentskim Newman-Keulsovim post-hoc testom. Snaga efekta je procijenjena s pomoću η2. Za interpretaciju korišteni su Cohenovi kriteriji: $\eta^2 = 0{,}02 – 0{,}13$ = mala snaga efekta, 0,13-0,26 = umjerena i > 0,26 = velika.

Ponovljivost mjerenja procijenjena je intraklasnim korelacijskim koeficijentom, a razlike u mjerenjima t-testom za zavisne uzorke. Upotrijebljen je statistički softver IMB SPSS 22 (IBM Corp, Armonk, SAD).

were taken for each wire and the result of each measurement was read two times.

Since metal corrosion in the oral cavity occurs as an electrochemical mechanism, wire corrosion was examined through the electrochemical method of cyclic polarization by using a PAR263A potentiostat and a frequency analyser PAR FRD 1025 (Princeton Applied Research, Oak Ridge, USA) (19). The measurements were performed in 300 ml of artificial saliva with addition of 6 probiotic tablets at a temperature of 37±2oC. Test wires were cut out and insulated with lacquer so that exposed surface was 0.61 cm2.

Polarisation curves of dependency of potential on logarithmic value of corrosion current density were used to determine corrosion current density, Icor, which is an equivalent to general corrosion rate i.e. speed at which a metal deteriorates. The incidence of local corrosion damage on the material was determined based on the shape of the polarisation curve, i. e. based on the ratio between corrosion potential, Ecor –the potential at which the lowest current density is recorded, passive oxide film breakdown potential Ebd –the potential at which a sudden increase in current density occurs, and repassivation potential Erp –the potential at which the reverse part of the curve shows that the currents become equal to those in the early stages of the cycle. At Ebd potential, the protective passive film becomes damaged and intensive dissolution of the alloy occurs, which can result in a significant release of nickel ions. The bigger the difference between Ecor and Ebd values, the lower the probability that significant damage to the passive film can occur under real conditions. Damaged passive film can be recovered (repassivated) at lower potential values in the reverse part of the polarisation curve. If repassivation takes place at potentials close to Ebd, the probability of occurrence of localised corrosion is negligible. Upon completion of electrochemical measurements, the surface of test samples was scanned by using a scanning electronic microscope with the secondary electron signal (Tescan Vega 3, Tescan, Brno, Czech Republic) at magnification 1000x.

In statistical analysis, t-test was used. Variance analysis with a Students-Newman-Keuls post-hoc test was also used. The effect size was assessed by η2. Cohen criteria were used for interpretation: η2=0.02-0.13 = small effect size, 0.13-0.26 = medium and >0.26 = large. Reproducibility of measurement was assessed by the intraclass correlation coefficient (ICC), and differences in measurements were determined by means of dependent t-test for paired samples. Statistical software IMB SPSS 22 (IBM Corp, Armonk, USA) was used.

Rezultati

Površinska hrapavost

Mjerenja površinske hrapavosti na dva mjesta na istoj žici upućuju na značajno slaganje u sva tri parametra (ICC = 0,693 – 0,875; $p < 0{,}001$) i izvrsnu ponovljivost očitanja pri mjerenju (ICC = 0,997 – 0,999; $p < 0{,}001$).

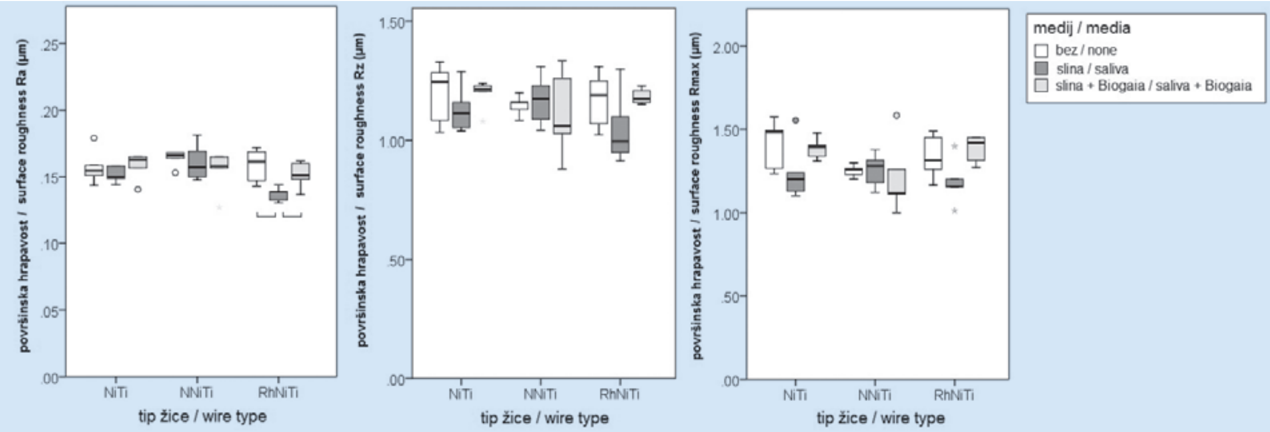
Nije bilo značajne razlike između nikal-titanijevih žica prije korozije. Izlaganje slini smanjuje Ra rodiranih žica (p = 0,015; η2 = 0,501; slika 1.). Medij značajno ne utječe na površinsku mikrogeometriju nitriranih i neobloženih žica.

Results

Surface roughness

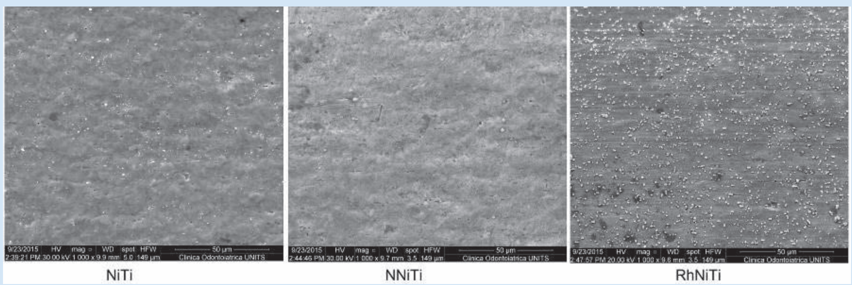
Measurements of surface roughness in two places of the same wire showed a significant correspondence of all three parameters (ICC=0.693-0.875; $p<0.001$) and an excellent reproducibility of measurement result readings (ICC=0.997-0.999; $p<0.001$).

The differences between NiTi wires before corrosion are not significant. Exposure to the saliva decrease Ra in rhodium coated wire (p=0.015; η2=0.501; Figure 1). Media do



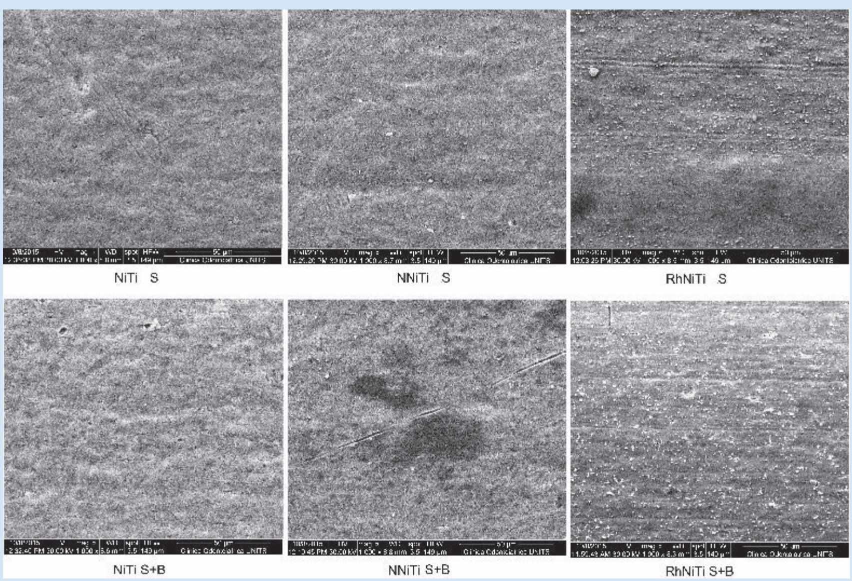
Slika 1. Usporedba površinske hrapavosti NiTi žica i eksperimentalnih uvjeta. Horizontalne linije povezuju medije koji stvaraju značajnu razliku za žicu iste vrste.

Figure 1 Comparison of surface roughness between NiTi archwire types and experimental conditions. Horizontal lines connect the media that produce significant differences for the same wire type.



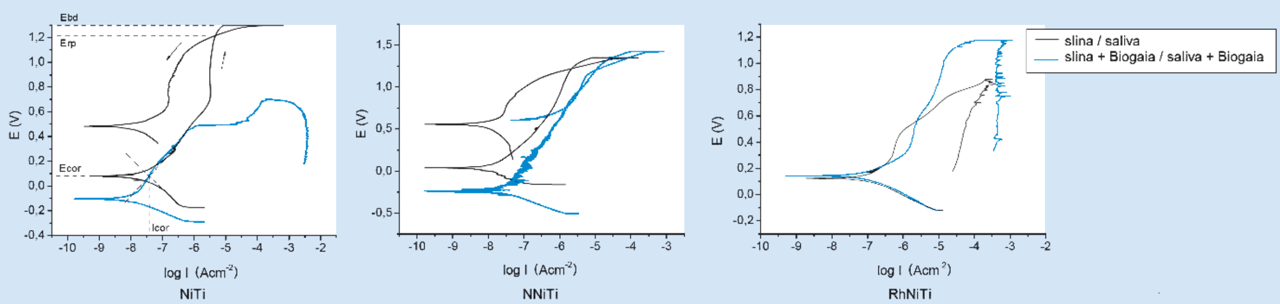
Slika 2. SEM, povećanje 1000x neeksponiranog materijala

Figure 2 SEM magnification 1000x of unexposed material



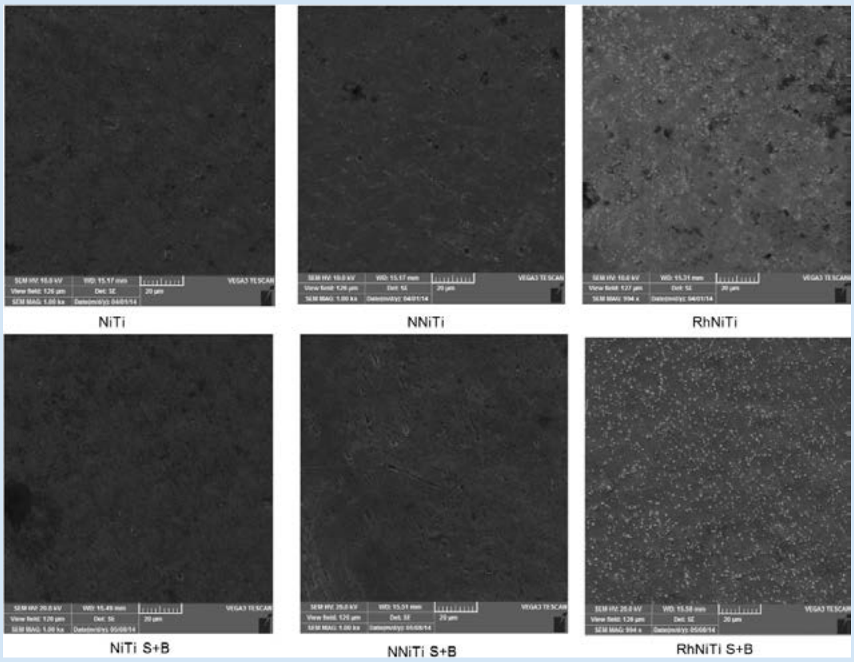
Slika 3. SEM, povećanje 1000x. Materijal nakon 28 dana izlaganja umjetnoj slini (prvi red) te umjetnoj slini i probiotičkom dodatku (drugi red).

Figure 3 SEM magnification 1000x material after being exposed for 28 days to artificial saliva (first row), and material after being exposed to artificial saliva and probiotic supplement (second row)



Slika 4. Grafički prikaz krivulja cikličkih polarizacijskih mjerenja NiTi žica u slini i slini s dodatkom probiotika

Figure 4 Graphic presentation of curves of cyclic polarisation measurements of NiTi archwires in saliva and in saliva containing probiotic supplement



Slika 5. SEM, povećanje 1000x. Neizložena žica (prvi red) te žica nakon elektrokemijskog testiranja u slini koja sadrži probiotik (drugi red).
Figure 5 SEM magnification 1000x of unexposed wire (first row) and wire after electrochemical testing in saliva containing probiotic supplement (second row)

SEM pri povećanju od 1000 puta potvrđuje nalaze profilometra (slike 2. i 3.). Uočava se da površina svih žica prije izlaganja nije potpuno homogena te da postoje mikrodefekti u strukturi površine, što je najizraženije na rodiranoj žici (slika 2.). Na pojedinim dijelovima vidljive su hrapavosti i mikropukotine u obliku zareza koje mogu biti posljedica mehaničkog oštećenja tijekom termocikliranja ili lokalizirane korozije. Nakon ekspozicije umjetnoj slini rodirana žica na pojedinim dijelovima izgleda nešto glađe (slika 3.).

Elektrokemija

Na slici 4. su polarizacijske krivulje ovisnosti potencijala o logaritamskoj vrijednosti gustoće struje korozije. Na žici neobloženoj nikal-titanijem mogu se uočiti pozitivnije vrijednosti korozijskog potencijala i potencijala pucanja pasivnoga filma u slini od onih zabilježenih u slini s probiotskim preparatom. I u slučaju čiste sline lagano nastaje repasivacija žica, što u slini s probiotikom nije slučaj. Kao i na neobloženim žicama, uzorci nitirirane površine u slini s probiotikom također imaju negativniji korozijski potencijal te slabije svojstvo repasivacije negoli u čistoj umjetnoj slini. Usporedbom rodirane žice u čistoj slini i u slini s probiotikom, uočava se da s dodatkom probiotika raste potencijal pucanja pasivnoga filma, ali nema mogućnosti repasivacije ni u jednoj otopini.

Usporedbom korozijskih parametara u umjetnoj slini i u slini s probiotikom uočava se da su, u odnosu na samu slinu, vrijednosti korozijskih struja u slini s probiotikom manje kad je riječ o žici neobloženoj nikal-titanijem, podjednake za nitriranu, a veće za rodiranu žicu. U slučaju neobložene žice, probiotik uzrokuje lokalnu koroziju. Na rodiranoj žici oštećenje nastaje malo teže negoli u čistoj slini, ali kada nastane zaštitni se sloj više ne može oporaviti. Na nitriranoj žici teže

not significantly affect surface microgeometry in nitrified and uncoated wires.

SEM at 1000x magnification confirms the results of profilometer (Figure 2 and 3). It was observed that the surface of all wires before exposure was not entirely homogenous and that there were microdefects in the surface structure, which was most pronounced in rhodium coated wire (Figure 2). Roughness and microcracks in the form of nicks were also observed in certain places, which could be a consequence of mechanical damage during thermocycling, or of localised corrosion. After exposition to artificial saliva, rhodium coated wire looked somewhat smoother in some places (Figure 3).

Electrochemistry

Figure 4 shows polarisation curves of dependency of potential on the logarithmic value of corrosion current density. In uncoated NiTi material immersed in saliva it is possible to observe more positive values of corrosion potential and passive film breakdown potential than those recorded in saliva containing probiotic supplement. Also, in the case of pure saliva, wire repassivation occurs easily, which is not the case with the saliva containing probiotic. As is the case with uncoated wire, nitrified surface samples in saliva containing probiotic also have a more negative corrosion potential and smaller repassivation ability than those immersed in pure artificial saliva. By comparing rhodium coated wire immersed in pure saliva and the wire immersed in saliva containing probiotic, one can note that addition of probiotic increases passive film breakdown potential, but there is no possibility of repassivation in solutions.

By comparing corrosion parameters in artificial saliva to those in saliva containing probiotic, we can see that the values of corrosion currents in saliva containing probiotic are lower in uncoated NiTi, equal in nitrified material and higher in rhodium coated wire than those in pure saliva. In uncoat-

Tablica 1. Vrijednosti korozivnih parametara
Table 1 Values of corrosion parameters

Žica • Archwire	Parametar korozije • Corrosion parameter	Medij • Media	Srednja vrijednost ± st. devijacija • Mean ± SD	95%-tni interval pouzdanosti • 95% CI*	p**	η2***
NiTi	Korozija strujne gustoće (Icor / nAcm^{-2}) • Corrosion current density (Icor / nAcm^{-2})	Slina • Saliva	31.3±16.3	-9.1-71.7		
		Probiotik • Probiotic	19.9±8.7	-1.6-41.4	0.343	0.224
	Korozijski potencijal (Ecor / mV) • Corrosion potential (Ecor / mV)	Slina • Saliva	76.3±20.4	25.6-127.0		
		Probiotik • Probiotic	-149.3±37.7	-243.0-(-55.7)	0.001	0.954
	Repasivacijski potencijal (Erp / mV) • Repasivation potential (Erp / mV)	Slina • Saliva	1205.0±11.5	1176.2-1233.8		
		Probiotik • Probiotic	-	-	-	-
	Potencijal slamanja (Ebd / mV) • Brakedown potential (Ebd / mV)	Slina • Saliva	1272.7±23.7	1213.9-1331.5		
		Probiotik • Probiotic	469.7±124.6	160.0-779.3	<0.001	0.968
NNiTi	Korozija strujne gustoće (Icor / nAcm^{-2}) • Corrosion current density (Icor / nAcm^{-2})	Slina • Saliva	24.3±20.5	-26.7-75.3		
		Probiotik • Probiotic	28.0±20.0	-21.8-77.8	0.834	0.013
	Korozijski potencijal (Ecor / mV) • Corrosion potential (Ecor / mV)	Slina • Saliva	-31.7±65.0	-193.1-129.8		
		Probiotik • Probiotic	-247.4±59.3	-394.7-(-100.0)	0.013	0.818
	Repasivacijski potencijal (Erp / mV) • Repasivation potential (Erp / mV)	Slina • Saliva	1193.0±29.8	1118.9-1267.1		
		Probiotik • Probiotic	729.9±14.9	692.9-767.0	<0.001	0.993
	Potencijal slamanja (Ebd / mV) • Brakedown potential (Ebd / mV)	Slina • Saliva	1328.7±82.1	1124.7-1532.6		
		Probiotik • Probiotic	1317.6±76.4	1127.8-1507.4	0.873	0.007
RhNiTi	Korozija strujne gustoće (Icor / nAcm^{-2}) • Corrosion current density (Icor / nAcm^{-2})	Slina • Saliva	100.7±40.1	1.2-200.2		
		Probiotik • Probiotic	120.4±12.7	88.8-152.1	0.461	0.142
	Korozijski potencijal (Ecor / mV) • Corrosion potential (Ecor / mV)	Slina • Saliva	162.0±34.0	77.6-246.4		
		Probiotik • Probiotic	137.0±3.7	127.9-146.1	0.273	0.287
	Repasivacijski potencijal (Erp / mV) • Repasivation potential (Erp / mV)	Slina • Saliva	-	-		
		Probiotik • Probiotic	-	-	-	-
	Potencijal slamanja (Ebd / mV) • Brakedown potential (Ebd / mV)	Slina • Saliva	764.7±60.4	614.5-914.8		
		Probiotik • Probiotic	1033.0±283.1	329.7-1736.3	0.184	0.392

* CI: interval pouzdanosti • confidence interval; p: razina značajnosti • level of significance; η2: veličina učinka • effect size.

nastaje oštećenje negoli u slini, ali kad nastane, oksidni se sloj nešto teže obnavlja (tablica 1.).

Na SEM snimkama uočava se na površini neobložene i nitrirane žice porast broja mikropukotina u obliku zareza nakon izlaganja umjetnoj slini s probiotskim preparatom, što se može povezati s pojavom lokalizirane korozije (slika 5.). Na rodiranoj žici zabilježeno je taloženje korozijskih produkata koji su vidljivi kao pravilno i gusto raspoređene bijele točke.

Rasprava

Naše istraživanje upućuje na to da i probiotički preparat koji se upotrebljava za oralnu primjenu u svrhu poboljšanja oralnoga zdravlja i održavanja povoljne mikrobne flore, u određenoj mjeri utječe na koroziju. Pretpostavka da će, uz korozivno djelovanje sline, probiotski preparat dodatno povećati opću i lokaliziranu koroziju nije u cijelosti točna. Na NiTi legurama s neobloženim površinama probiotski preparat uzrokuje veću sklonost prema lokaliziranoj koroziji, no manju prema općoj koroziji. Opća korozija najčešća je vrsta koja pogađa sve metale, a pojavljuje se na cijeloj površini metala (20). Metal podliježe reakcijama oksidacije i redukcije u

ed wire, probiotic causes local corrosion. In rhodium coated wire, damage is less likely to occur than in pure saliva, but once it does occur, the protective layer cannot recover any more. In nitrified wire, damage is less likely to occur, but once it does, it is more difficult for the oxide layer to recover (Table 1).

SEM scan shows an increase in the number of microcracks in the shape of a nick on the surface of uncoated and nitrified wire after exposure to artificial saliva containing probiotic supplement, which can be linked to localised corrosion (Figure 5). In rhodium coated wire, precipitation of visible corrosion products has taken place, and one can also observe densely distributed white spots.

Discussion

Our research shows that probiotic supplement used orally for the purpose of improving oral health and maintaining favorable microbial flora influences corrosion to some extent. It was assumed that, in addition to the corrosion effect of saliva, probiotic supplement would further increase general and localised corrosion. However, this assumption is not entirely correct. In NiTi alloys with uncoated surface, probiotic supplement leads to a greater propensity for localised corrosion and smaller propensity to general corrosion. General corrosion is the most common type of corrosion. It affects all metals and develops on the entire metallic surface (20). Metal is mediju koji ga okružuje, a ovisno o vrsti metala korozija će biti različitog intenziteta. Lokaliziranu koroziju obilježava to da je intenzitet korozije pojačan na lokalnoj razini. Pojavit će se ako postoji nehomogenost u sastavu materijala ili okoline. Granice zrna u metalu mogu biti mjesto gdje počinje korozija zbog svojeg stanja povišene energije. Pukotine su također osjetljive na koroziju, s obzirom na to da je kemijski sastav u njima drukčiji od okolnog medija.

Usna šupljina idealno je okružje za razvoj korozije, a već je istaknuto da određene bakterijske vrste mogu uzrokovati koroziju titanijevih materijala (21 – 23). I naše istraživanje upućuje na to da bi pojava lokalizirane korozije mogla biti potaknuta probiotičkim bakterijama iz preparata Biogaia. Možda je ona posljedica apsorpcije i probavljanja metala iz legure što čine bakterije, ili taloženja netopivih komponenti pastile na ortodontsku žicu pri čemu nastaje korozija ispod naslaga. Istraživanje izloženosti nikal-titanijeve legure samoj kulturi *Lactobacillus reuteri* potvrdilo bi u kojoj mjeri ta bakterija uzrokuje koroziju.

Konstrukcija ortodontske naprave omogućuje idealne uvjete za adheziju bakterija te razvoj biofilma u kojemu nastaje složen mehanizam interakcije između aerobnih i anaerobnih bakterija, što pogoduje nastanku korozije. Korozivno djelovanje probiotskih preparata i *L. reuteri* na ortodontske lukove dosad nije istraženo. Ni mehanizam djelovanja probiotika nije potpuno objašnjen, ali dokazano je da oba sprječavaju adheziju patoloških bakterija na površine, mijenjaju pH okoline, a time i uvjete za život patogenih mikroorganizama, izlučuju antimikrobne tvari te utječu na imunosni odgovor domaćina (24). Upravo promjene uvjeta u okolišu, zbog metaboličkih produkata probiotskih bakterija, drugi je mogući način nastanka korozije na nikal-titanijevoj leguri.

Jedna od hipoteza bila je da će korozivno djelovanje probiotskog preparata biti vidljivo kao povećana površinska hrapavost neobložene nikal-titanijeve žice. Istraživanja su dokazala povezanost korozije slitina i povećanja površinske hrapavosti, što neposredno utječe na mehanička svojstva žica te može rezultirati pucanjem ortodontske naprave (25). Površinska hrapavost utječe na koeficijent trenja, što je bitan čimbenik u ortodontskom pomaku zuba (26 – 28). Pojavljuje se pri sklizanju žice kroz bravicu. Što je trenje između bravice i žice veće, više sile troši se na svladavanje otpora trenja, što u konačnici usporava pomak zuba i produljuje ortodontsku terapiju. No, suprotno našoj pretpostavci, parametri mikrogeometrijske nepravilnosti površine nisu se značajno promijenili nakon izlaganja nikal-titanijevih žičanih lukova slini i probiotskom preparatu.

Očekivali smo da će vrsta obloge promijeniti sklonost prema koroziji te da će nitriranje površine smanjivati, a rodiranje povećavati tu sklonost. Kako smo i pretpostavili, ovo istraživanje potvrđuje da oblaganje površine mijenja sklonost prema koroziji. Općenito, nitriranje površine poboljšava korozijsku otpornost, a rodiranje je smanjuje. Probiotski preparat utječe i na opću i na lokaliziranu koroziju, a utjecaj je modificiran vrstom obloge. Na rodiranoj žici vidljivo je povećanje i opće korozije i pojave lokalizirane korozije nakon primjene probiotskog preparata. Na neobloženoj žici probiotski preparat smanjuje opću koroziju, no povećava lokalziranu. U

susceptible to oxidation and reduction reactions in the medium surrounding it, and, depending on the type of metal, corrosion will vary in intensity. Local corrosion is characterized by corrosion intensity being increased at the local level. It will take place if there is a nonhomogeneity in the composition of the material or of the environment. Grain boundaries in metal can be the places where corrosion starts because of their state of elevated energy. Cracks are also sensitive to corrosion considering that chemical composition in a crack is different than the one in the surrounding medium.

The oral cavity is an ideal environment for the development of corrosion, and it has been previously reported that certain species of bacteria could cause corrosion of materials containing titanium (21-23). Our research also showed that appearance of local corrosion could be promoted by the presence of probiotic bacteria from the BioGaia supplement. It is possible that corrosion is a consequence of absorption and digestion of metal from the alloy by bacteria, or of precipitation of insoluble components from the pastille on orthodontic archwire, in which case corrosion occurs under the precipitate. A research on the exposure of NiTi alloy to Lactobacillus reuteri culture could determine to what extent the bacterium itself is the cause of corrosion.

The structure of an orthodontic appliance provides perfect conditions for bacterial adhesion and development of a biofilm in which a complex mechanism of interaction between aerobic and anaerobic bacteria takes place, which is suitable for the occurrence of corrosion. Corrosive effect of probiotic supplements and L. reuteri on orthodontic arches has not been researched yet. Also, the mechanism of probiotic' action has not been fully explained, but it has been proven that probiotics prevent adhesion of pathological bacteria onto surfaces, change pH of the environment and the living conditions of pathogenic microorganisms, secrete antimicrobial substances and affect the host's immunological response (24). The products of metabolism of probiotic bacteria could change environmental conditions and that could be another possible way of corrosion of a NiTi alloy (4).

It has also been hypothesized that corrosive effect of probiotic supplements would manifest itself in the form of an increased surface roughness of uncoated NiTi wire. A correlation between corrosion of alloys and an increase in surface roughness, which directly affects mechanical properties of wires and can lead to an orthodontic wire fracture, has been proven by research (25). Surface roughness affects the friction coefficient, which is an important factor of orthodontic tooth movement (26-28). Friction occurs as the wire slides through a bracket. The greater the friction between a bracket and the wire, the more force is required to overcome friction resistance, which eventually slows down tooth movement and extends the orthodontic therapy. But, contrary to our assumption, the parameters of micro geometric irregularities of the surface did not significantly change after exposing NiTi orthodontic archwires to saliva and probiotic supplement.

We had expected that the type of coating would change the propensity for corrosion and that surface nitrification would decrease, whereas rhodium coating would increase propensity for corrosion. As we had anticipated, this research slučaju nitrirane žice probiotik neće u većoj mjeri mijenjati ni opću ni lokaliziranu koroziju u odnosu na samu slinu. Na temelju dobivenih rezultata može se zaključiti da su ispitivani preparati najsigurniji za primjenu na nitriranim žicama, a za neobložene žice postoji velika mogućnost da se pojave lokalizirana korozijska oštećenja koja bi potaknula otpuštanje iona nikla u usnu šupljinu. Dosadašnja istraživanja na ovim vrstama žica pokazala su iste rezultate te je smanjena korozijska otpornost rodiranih žica objašnjena pojavom mikrogalvanskih članaka između plemenite prevlake i manje plemenite podloge (NiTi legure) koji se pojavljuju zbog defekata u samoj prevlaci (29).

Iako smo pretpostavili da će korozivno djelovanje probiotskog preparata utjecati na povećanje površinske hrapavosti svih ispitivanih žica u odnosu na djelovanje same sline, nije pronađena značajna razlika u površinskoj hrapavosti nitrirane i rodirane žice nakon izlaganja slini i izlaganja djelovanju probiotskog preparata. Medij, kao ni vrsta obloge, ne utječu značajno na površinsku hrapavost nitrirane i rodirane NiTi žice, iako je u dosadašnjim istraživanjima uočena povećana površinska hrapavost rodiranih NiTi žica u odnosu na neobložene NiTi žice te NiTi žice s polimernom prevlakom (30, 31).

Zaključak

Probiotički dodatak utječe na opću i lokaliziranu koroziju ovisno o tipu prevlake NiTi žice. Povećava opću koroziju rodirane žice te uzrokuje lokaliziranu koroziju neobložene i rodirane žice. Nitrifikacija površine poboljšava otpornost, a rodiranje je pogoršava. Probiotički dodatak ne utječe značajno na hrapavost površine u usporedbi s a slinom.

Sukob interesa

Nije bilo sukoba interesa.

Zahvala

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confirmed that surface coating does change propensity for corrosion. Generally, nitrification improves, and rhodium coating decreases corrosion resistance. Probiotic supplement affects both general and localised corrosion and the effect is modified by the type of coating. In rhodium coated wire, an increase in both general and localised corrosion has been observed after probiotic supplement had been used. In uncoated wire, probiotic supplement decreases general corrosion but increases localised corrosion. In nitrified wire, probiotic did not change general or localised corrosion to a large extent when compared to the effect of saliva alone. Based on the results obtained, one can conclude that the tested probiotic supplements are safest for use with nitrified wire, while in the case of uncoated wire, there is a significant possibility of localised corrosive damage which would lead to nickel ions being released into the oral cavity. Previous research conducted on these types of wire showed similar results and reduced corrosion resistance of rhodium coated wires was explained through existence of micro galvanic cells between the noble coating and the less noble underlying surface (NiTi alloy), which occurs due to defects in the coating itself (29).

Although we had assumed that corrosive effect of probiotic supplement would result in an increase in surface roughness of all the tested wires when compared to the effect of saliva alone, no significant difference in surface roughness of nitrified and rhodium coated wire was found after exposing the wire to saliva versus exposing it to probiotic supplement. Neither the medium nor the type of coating have significant effect on surface roughness of nitrified and rhodium coated wire, although previous research showed increased roughness of rhodium coated and polymer coated NiTi wires when compared to uncoated NiTi (30, 31).

Conclusion

Probiotic supplement affects general and local corrosion depending on the type of coating of the NiTi archwire. It increases general corrosion of rhodium coated wire and causes localised corrosion of uncoated and rhodium coated archwire. Surface nitrification improves corrosion resistance, while rhodium coating decreases it. Probiotic supplement does not have greater influence on surface roughness compared to that of saliva.

Conflict of interest

None declared

Aknowledgement

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Abstract

Objectives: The aim of the study was to examine how probiotic supplements affect the corrosion stability of orthodontic archwires made of nickel-titanium alloy (NiTi). **Materials and Methods**: NiTi archwires (0.508x0.508 and having the length of 2.5 cm) were tested. The archwires (composition Ni=50.4%, Ti=49.6%) were uncoated, nitrified and rhodium coated. Surface microgeometry was observed by using scanning electron microscope and surface roughness was measured by profilometer through these variables: roughness average, maximum height and maximum roughness depth. Corrosion was examined by electrochemical method of cyclic polarisation. **Results**: Rhodium coated alloy in saliva has significantly higher general corrosion in saliva than nitrified alloy and uncoated alloy, with large effect size ($p=0.027$; $\eta 2=0.700$). In the presence of probiotics, the result was even more pronounced ($p<0.001$; $\eta 2=0.936$). Probiotic supplement increases general and localised corrosion of rhodium coated archwire and slightly decreases general corrosion and increases localised corrosion in uncoated archwire , while in the case of nitrified archwire the probability of corrosion is very low. The differences in surface roughness between NiTi wires before corrosion are not significant. Exposure to saliva decreases roughness average in rhodium coated wire ($p=0.015$; $\eta 2=0.501$). Media do not significantly influence surface microgeometry in nitrified and uncoated wires. **Conclusion**: Probiotic supplement affects corrosion depending on the type of coating of the NiTi archwire. It increases general corrosion of rhodium coated wire and causes localised corrosion of uncoated and rhodium coated archwire. Probiotic supplement does not have greater influence on surface roughness compared to that of saliva.

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