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Otpuštanje iona titanija iz inovativnog titanij-magnezijeva kompozita: istraživanje *in vitro*

Titanium Ions Release from an Innovative Titanium-Magnesium Composite: an in Vitro Study

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Sažetak

Svrha: U ovom radu istraživala su se korozijska svojstva inovativnog titanij-magnezijeva (Ti-Mg) kompozita proizvedenog metodom metalurgije praha (P/M). **Materijal i metode:** Ispitivane su dvije grupe eksperimentalnog materijala – s 1 masenim udjelom (mass% Ti-1Mg) i 2 masena udjela (mass% Ti-2Mg) magnezija u titanijskoj osnovi te su uspoređene s komercijalno čistim titanijem (CP Ti). Test uranjanja i kemijska analiza četiriju otopina: umjetne sline, umjetne sline pH 4, umjetne sline s dodatkom fluora i Hankove otopine, provedeni su nakon 42 dana uranjanja metodom masene spektrometrije induktivno spregnutom plazmom (ICP – MS) kako bi se ustanovila količina otpuštenih iona titanija (Ti). Za određivanje svojstava površine korištene su analize SEM i EDS. **Rezultati:** Razlika u rezultatima između različitih ispitivanih otopina procjenjivana je ANOVA-om i Newman-Keulsovim testom na razini značajnosti od $p < 0,05$. Utjecaj prediktorskih varijabli utvrđivan je multiplom regresijskom analizom. Rezultati ovog istraživanja pokazuju nisku stopu korozije titanija u ispitivanoj skupini Ti-Mg. Uočeno je do 46 puta, odnosno 23 puta manje otapanje iona titanija iz Ti-1Mg i Ti-2Mg u usporedbi s kontrolnom skupinom. Između ispitivanih otopina, umjetna slina s dodatkom fluora pokazala je najveći korozijski učinak među svim ispitivanim uzorcima. SEM-analiza pokazala je sačuvanu dvofaznu strukturu površine, a EDS-analiza upozorila je na moguća bioaktivna svojstva površine. **Zaključak:** Ti-Mg kompozit proizveden metodom P/M-a sugerira se kao materijal boljih korozijskih svojstava u usporedbi s čistim titanijem (CP Ti).

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titanij; magnezij; korozija; uranjanje; umjetna slina; izmjena iona

Uvod

Unatoč dugogodišnjoj upotrebi titanija kao biomaterijala dobre biokompatibilnosti (1), u novijoj znanstvenoj literaturi sve je više podataka o njegovim neželjenim korozijskim svojstvima (2 – 4). Osnovno obilježje korozije titanija jest pasivacija, tj. formiranje zaštitnoga pasivnog dioksidnog sloja (5) koji štiti kovinu od daljnje korozije. No, takav površinski sloj ima slabu zaštitnu ulogu u uvjetima s niskom pH vrijednošću (3, 6) i u prisutnosti fluora (F) (7, 8). Nizak pH svojstven je u upalnim uvjetima (9) koji mogu odgovarati onima u kirurškoj rani neposredno nakon implantacije (10). Nizak pH može se također izmjeriti u usnoj šupljini u kojoj je velika količina biofilma i visoka metabolična aktivnost kariogenih bakterija (11, 12). Fluor je sveprisutan u proizvodima za

Introduction

Despite long-standing use of titanium as a biomaterial of good biocompatibility (1), there is a certain number of papers in recent scientific literature reporting its undesirable corrosion properties (2-4). The main corrosion characteristic of titanium is the passivation i.e. the formation of a highly protective passive dioxide layer (5) that protects the metal from further corrosion. However, such a protective surface layer shows poor protective function in low pH value conditions (3, 6) and in the presence of fluoride (F) (7, 8). The low pH value is typical of inflammatory condition (9) that may correspond to the condition of surgical wound immediately after implantation (10). Also, low pH value may be found in the oral cavity with a large amount of biofilm and high održavanje oralne higijene, kao što su zubne paste i vodice za ispiranje usta.

Posljedica korozije titanijskog biomaterijala jest otpuštanje iona titanija (Ti) u okolno područje. Mine i suradnici su, na kulturi stanica, pokazali da ioni titanija mogu interferirati s procesom diferencijacije osteoblasta i osteoklasta te štetno utjecati na cjelokupan proces cijeljenja i oseointegracije (13). Slični su bili rezultati istraživanja Wachia i suradnika na životinjskom modelu. Naime, oni su ustanovili da nakupljeni ioni titanija u okolnom vezivnom i gingivnom tkivu mogu potaknuti infiltraciju monocita, proizvodnju citokina i, posljedično, diferencijaciju osteoklasta, što može voditi u klinički značajnu resorpciju alveolne kosti (14). Istraživanje Baraa i suradnika pokazalo je da korodirana površina lakše nakuplja patogene bakterije, što potiče upalne promjene oko implantata (15). Dugogodišnja upotreba titanija istaknula je problem preosjetljivosti i alergije na titanij, što može rezultirati neuspjehom u terapiji dentalnim implantatima. Iako su već provedena neka klinička istraživanja o temi alergije na titanij (16), većina autora slaže se da se zasad još ne zna dovoljno o takvim komplikacijama te da je, radi boljeg razumijevanja, potrebno više podataka temeljenih na dokazima, a nužno je provesti i detaljnija longitudinalna klinička istraživanja (2, 17).

Magnezij je posljednjih godina u žarištu znanstvenog zanimanja zbog povoljnog učinka na proces oseointegracije (18 – 20). Kao esencijalni makroelement ljudskog organizma, magnezij sudjeluje u važnim energijskim procesima unutar stanice (21) te potiče diferencijaciju pluripotentnih stanica, proliferaciju i migraciju osteoblasta (22). Galli i suradnici ustanovili su da magnezij inducira gensku ekspresiju osteogenih biljega te osteokondukciju (23). Castellani i suradnici su, na životinjskom modelu, dokazali da taj element, za razliku od titanija, postiže veću dodirnu površinu na sučelju implantat – kost i bolju mehaničku čvrstoću bez pojave sustavnoga upalnog odgovora (24). Ipak, zbog svojih biodegradabilnih svojstava, velike kemijske reaktivnosti i nepovoljnoga korozijskog ponašanja, čisti magnezij se, kao dentalni biomaterijal, može koristiti samo u kombinaciji s drugim kovinskim biomaterijalima (25).

Metalurgija praha (P/M) tehnološki je pristup koji se primjenjuje u procesu proizvodnje titanijskih biomaterijala kako bi se, između ostaloga, postigla bolja mehanička svojstva materijala i smanjila cijena proizvodnog procesa (26). Nepoželjni učinci dugogodišnjeg korištenja konvencionalno proizvedenoga titanijskog biomaterijala (27, 28) sugeriraju potrebu za novom strategijom u proizvodnji biomaterijala. P/M može biti način kako poboljšati mehanička svojstva i smanjiti troškove, ali i postići takav biološki odgovor koji se može nazvati biomimetičkim (29, 30). P/M-postupak sastoji se od miješanja prahova kovina, homogenizacije, zbijanja i konsolidacije prikladnim tehnološkim procesima i konačno od sinteriranja kako bi se čestice međusobno povezale (31). U ovom istraživanju, titanij-magnezijev kompozit (Ti-Mg) proizveden je miješanjem praha titanijske osnove s 1 i 2 masena udjela (mass%) magnezija (Mg).

Moguće tehničko rješenje za poboljšanje korozijske otpornosti titanijskih biomaterijala jest uvođenje alternativ-

metabolic activity of cariogenic bacteria (11, 12). Fluoride is ubiquitous in oral hygiene products such as toothpastes and mouthwashes.

The consequence of the titanium biomaterial corrosion is the release of titanium ions (Ti) in the surrounding area. Mine et al. have shown on the cell culture that Ti could interfere with the process of differentiation of osteoblasts and osteoclasts and adversely affect the whole process of healing and osseointegration (13). Similarly, the experiments made by Wachi *et al.*, on the animal model, suggested that accumulated Ti in the surrounding connective and gingival tissue might induce the infiltration of monocytes, the production of cytokines and consequently, the differentiation of osteoclast cells that could lead to clinically significant alveolar bone resorption (14). The study of Barao *et al.* showed that the corroded surface more easily accumulated the pathogenic bacteria that could lead to inflammatory changes around the implant (15). Due to long-term application of titanium-based dental implants, the problem of hypersensitivity and allergy to titanium which could provoke dental implant failure is emphasized. Although some clinical trials on the topic of titanium allergy have already been carried out (16), most authors agree with the fact that so far there are still not enough data on such complications and that, for better understanding, it is necessary to have more evidence-based data and to perform more detailed longitudinal clinical studies (2, 17).

Magnesium (Mg) has recently been in the focus of scientific interest due to its favorable effect on the process of osseointegration (18-20). As the essential macro element of the human body, Mg participates in important intracellular energy processes (21) and stimulates the differentiation of pluripotent cells, proliferation and migration of osteoblasts (22). Galli et al. demonstrated that the presence of Mg promoted gene expression of osteogenic markers and improved osteoconduction (23). Castellani et al., in an animal model, showed that Mg obtained greater contact surface of the implant-bone interface and better mechanical strength without the occurrence of systemic inflammatory response in contrast to titanium (24). However, due to its biodegradable properties, high chemical reactivity and unfavorable corrosion behavior, pure Mg as a dental biomaterial can be used only in combination with other metallic biomaterials (25).

Powder metallurgy (P/M) is a technological approach used for titanium-based biomaterials processing in order, among other things, to improve the mechanical properties of materials and to reduce the price of the production process (26). Adverse effects of long-term applied titanium biomaterials (27, 28) produced by conventional ways point to the need for a new strategy in the production of biomaterials. P/M can be a way to improve not only mechanical properties and to reduce the costs but also to achieve such a biological response that can be denominated as biomimetic (29, 30). The procedure of P/M consists of mixing metal powders, homogenization, compacting and consolidation through a suitable technological process and finally sintering to bond powder particles (31). In the present study, titanium-magnesium composite (Ti-Mg) was prepared by mixing powders of titanium matrix with 1 and 2 mass% of Mg.

ne proizvodne tehnologije, tj. metalurgije praha. Svrha ovog istraživanja je istražiti korozijsko ponašanje inovativnoga titan-magnezijeva (Ti-Mg) materijala i usporediti ga s komercijalno čistim titanijem (CP Ti). Ispitivane otopine korištene su za simuliranje bioloških uvjeta (usne šupljine, kosti). Pretpostavlja se da količina magnezija u titanijskoj osnovi značajno utječe na korozijsko ponašanje ispitivanog materijala. Pretpostavlja se da su fluor i niska pH vrijednost značajni korozivni čimbenici okoliša koji utječu na ispitivani materijal.

Materijali i postupci

Priprema materijala

Materijal koji se koristio u ovom istraživanju (Ti-1Mg i Ti-2Mg) proizveden je tehnologijom P/M-a. Prah 99,4 postotnog α-titanija, proizvedenog hidrid-dehidridnim postupkom (HDH), prosječne veličine čestica < 150 µm, pomiješan je s 1, odnosno 2 masena udjela atomiziranoga praha 99,8 posto magnezija prosječne veličine čestica od 30 µm. Miješanje prahova obavljalo se 30 minuta miješalicom Turbula (GlenMills, Clifton, SAD). Nakon homogenzacije prahova, praškasta mješavina zbijena je postupkom hladnoga izostatičkog prešanja (CIP) pod 200 MP-a. CIP sirovci tada su se prešali uniaksijalnim prešanjem u vakuumu (VP) na 500 °C i 1300 MP-a u potpuno čvrsti titan-magnezijev kompozit. Komercijalno čisti titanij 4. stupnja za biomedicinsku upotrebu (CP Ti 4) (Acnis International, Francuska) proizveden konvencionalnim postupkom lijevanja i vrućeg valjanja, korišten je kao kontrolna skupina u obliku šipke. Uzorci su podijeljeni u dvije skupine, pa je ispitivana skupina sadržavala sljedeće uzorke: Ti-1Mg i Ti-2Mg, a kontrolna CP Ti 4. Svi materijali tada su razrezani erozimatom sa žicom (wire EDM). Pripremljeno je dvanaest uzoraka od svakog materijala u obliku diska promjera 27 mm i debljine 4 mm. Površine uzoraka obrađene su standardnim metalografskim postupcima – brušene su i polirane silikonsko-karbidnim papirom pod mlazom vode (u skladu s normom ISO 6344-1) gradacije od #320 – 4000 na stroju za brušenje/poliranje (Phoenix Alpha, Buehler, SAD) na 60 o/min.

Ispitivane otopine

U sklopu ovog istraživanja korištene su četiri vrste otopina za uranjanje uzoraka:

a) umjetna slina modificirana prema Fusayami (AS) (sadržavala je 0,4 g NaCl, 0,4 g KCl, 0,795 g $CaCl_2 \cdot 2H_2O$, 0,69 g $NaH_2PO_4 \cdot 2H_2O$, 0.005 g $Na_2S \cdot 9H_2O$ i 1.0 g uree u 1000 ml deionizirane vode);
b) umjetna slina modificirana prema Fusayami pH vrijednosti 4 koja je pripremljena tako da je u AS dodana mliječna kiselina ($C_3H_6O_3$) i pH vrijednost dovedena do 4;
c) umjetna slina modificirana prema Fusayami s dodatkom fluora (F) koja je pripremljena tako da je u AS dodano 0,2 masenog udjela natrijeva fluorida (NaF); Hankova otopina (HBSS) (sadržavala je 8,0 g NaCl, 1,0 g d-glukoze, 0, g KCl, 0,35 g $NaHCO_3$, 0,14 g $CaCl_2$, 0,098 g $MgSO_4 \cdot 7H_2O$, 0,6 g KH_2PO_4, 0,048 g Na_2HPO_4 u 1000 ml dei-

A possible technical solution for improvement of corrosion resistance of Ti-based biomaterials is introduction of an alternative production technology i.e. powder metallurgy. The aim of the present study was to explore the corrosion behavior of an innovative Ti-Mg material and to compare it to commercially pure titanium (CP Ti). Test solutions were used to simulate biological environment (e.g., mouth, bone). It is assumed that the amount of Mg in the titanium matrix significantly affects the corrosion behavior of tested material. Fluoride and low pH value of the surrounding media are hypothesized as significantly corrosive environmental factors for the material in question.

Materials and methods

Materials preparation

Materials used in this research (Ti-1Mg and Ti-2Mg) were produced by means of P/M technology. The 99.4% α-titanium powder produced by hydrid-dehydrid (HDH) technology, with the median particle size of <150 µm, was mixed with 1 or 2 mass% of atomized 99.8% Mg powder, with the median particle size of 30 µm, respectively. Blending of powder mixtures was performed for 30 min using Turbula mixer, GlenMills, Clifton, USA. After the homogenization of powders, the loose powder mixtures were cold compacted by cold isostatic pressing (CIP) at 200 MPa. CIP powder billets were compressed by uniaxial vacuum pressing (VP) at 500 °C and 1300 MPa in fully consolidated sound Ti-Mg composite materials. Commercially pure titanium grade 4 for biomedical use (CP Ti 4), Acnis International, France, produced by conventional process of casting and hot rolling was used for the control group of specimens in the as-received form of a bar. The samples were divided into two groups: test group containing specimens of Ti-1Mg and Ti-2Mg; and the control group containing CP Ti 4. All materials were then cut using the wire electrical discharge machining (wire EDM) technique. Twelve specimens of each material were prepared in the discs shape with the 27 mm diameter and 4 mm thickness. The specimen surfaces were prepared using standard metallographic methods, ground and polished using wet silicon-carbide paper (according to ISO 6344-1) from #320 - 4000 successively, on a grinder/polisher Phoenix Alpha, Buehler, USA, at 60 rpm.

Test solutions

In this study, four testing solutions for immersion of specimens were used:

a) Modified Fusayama's artificial saliva (AS) (contained 0.4 g of NaCl, 0.4 g of KCl, 0.795 g of $CaCl_2 \cdot 2H_2O$, 0.69 g of $NaH_2PO_4 \cdot 2H_2O$, 0.005 g of $Na_2S \cdot 9H_2O$ and 1.0 g urea in 1000 ml of deionized water);
b) Modified Fusayama's artificial saliva-pH 4 prepared by adding lactic acid ($C_3H_6O_3$) to AS and adjusting the pH value to 4;
c) Modified Fusayama's artificial saliva with F prepared by adding 0.2 mass% sodium fluoride (NaF) to AS;
d) Hank's balanced salt solution (HBSS) (contained 8.0 g of NaCl, 1.0 g of d-glucose, 0.4 g of KCl, 0.35 g of $NaHCO_3$, 0.14 g of $CaCl_2$, 0.098 g of $MgSO_4 \cdot 7H_2O$, 0.06 g of KH_2PO_4, 0.048 g of Na_2HPO_4 in 1000 ml deionized

onizirane vode). Sve kemikalije bile su čistoće analitičkoga stupnja. Prema normi ISO 3696, deionizirana voda kojom smo se koristili u ovom istraživanju bila je stupnja 2. Sve otopine pripremljene su neposredno prije uranjanja.

Statički test uranjanja

Statički test uranjanja proveden je u skladu sa sadašnjom normom za korozijska ispitivanja kovinskih materijala u dentalnoj medicini ISO 1027:2011. Na početku ispitivanja mikrometarskom mjerkom izračunata je površina svakog uzorka (u cm^2). Uzorci su prije uranjanja dvije minute ultrazvučno očišćeni u etanolu, isprani deioniziranom vodom i osušeni stlačenim zrakom bez primjesa vode i ulja (u skladu s normom ISO 7183). Uzorci su ispitivani u triplikatu za svaku vrstu materijala i svaku vrstu otopine. Svaki uzorak stavljen je nemetalnom hvataljkom u zasebnu, volumetrijski označenu bočicu od borosilikatnog stakla (u skladu s normom ISO 1042) i potopljen u svježe pripremljenu otopinu tako da dodiruje površinu bočice minimalnom površinom koja je potrebna za podupiranje te tako da je potpuno potopljen. U dodatnom paralelnom setu postavljene su bočice za *slijepu probu* u triplikatu, bez uzorka i ispitivane su na identičan način. Prije i poslije uranjanja, pH vrijednost i volumen svake otopine izmjereni su pH-metrom (HQ440d Multi-Parameter, Hach, SAD). Sve bočice zatvorene su kako bi se spriječilo isparavanje. Ispitivanje je provedeno na 37 ºC u termostatu. Uzorci su bili uronjeni 42 dana. Nakon razdoblja uranjanja izvađeni su, isprani deioniziranom vodom i pažljivo osušeni. Uzete su ispitivane otopine i prenesene u polipropilenskim bočicama na kemijsku analizu.

Masena koncentracija otopljenih iona titanija (µg/l) izmjerena je metodom masene spektrometrije induktivno spregnutom plazmom (ICP-MS) na masenom spektrometru Elan 9000 (Perkin-Elmer, SAD) s automatskim sustavom za uzimanje uzorka Perkin-Elmer AS 93 plus (Perkin-Elmer, SAD) tijekom triju ponavljanja u sljedećim uvjetima: protok plina kroz raspršnu komoru 0,9 l/min, snaga ICP-RF 1000 W, napon leća 7,75 V, napon analogne faze -1887 V, napon pulsne faze 1100 V, granica detekcije 65 V i AC rod *offset* -2,9 V. Pripremljena je serija kalibracijskih otopina kako bi se odredila masena koncentracija otopljenih iona titanija s pomoću norme Perkin-Elmer Pure Plus Multi-Elemental Calibration Standard 5 (Perkin-Elmer, SAD).

Količina otpuštenih iona titanija za svaki uzorak (µg/cm^2) izračunata je iz podataka o masenoj koncentraciji u ispitivanim otopinama, otopinama *slijepe probe* i površine uzoraka koristeći se formulom: količina otpuštenih Ti (µg/cm^2) = volumenu ispitivane otopine (l) · (masena koncentracija Ti u ispitivanoj otopini (µg/l) – srednja vrijednost masenih koncentracija Ti *slijepih proba* računata iz triju bočica (µg/l)) / površinu uzorka (cm^2). Količina otopljenih iona titanija uzimala se kao nula kada je njihova koncentracija u ispitivanoj otopini bila niža od one u *slijepoj probi.* Srednje vrijednosti i standardne devijacije računate su za tri bočice.

Analiza površine

Analiza površine uzoraka provedena skenirajućim elektronskim mikroskopom (SEM) (tip VEGA TS5136LS, Tescan, Republika Češka), koristeći se detektorima SE i BSE na povećanju od 50 i 200 puta te pod naponom ubrzanja elektronskog

water). All chemicals were of analytical grade. According to the ISO 3696 deionized water used in this research was grade 2. All solutions were prepared freshly prior to the immersion.

Static immersion test

The static immersion test was carried out in accordance with the currently specified standard for corrosion test of metallic materials in dentistry ISO 10271:2011. Surface area (in cm^2) of each specimen was measured using a micrometer gauge at the start of the experiment. The specimens were cleaned ultrasonically for 2 minutes in ethanol, rinsed with deionized water and dried with oil- and water-free compressed air (according to the standard ISO 7183) prior to immersion. The samples were set in triplicates for each material and each type of solution. Each specimen was placed in its individual volumetric flask made of borosilicate glass (according to the standard ISO 1042), using metal-free pincers, immersed in freshly prepared solution such that it did not touch the flask surface except in a minimum support line and that it was completely covered by the solution. In additional parallel sets, the flasks of blank tests in triplicates without samples were set and these were treated in identical manner. pH value and volume of each test solution were measured before and after immersion period using pH-meter HQ440d Multi-Parameter Meter, Hach, USA. All flasks were closed to prevent evaporation. The experiment was performed at 37 ºC in thermostat. The immersion was carried out for 42 days. After the period of immersion, the specimens were taken out, rinsed with deionized water and gently dried. Test solutions were taken and transferred in polypropylene tubes for chemical analysis.

The mass concentration of dissolved Ti (µg/l) was measured using inductively coupled plasma mass spectrometry (ICP-MS) on mass spectrometer Elan 9000, Perkin-Elmer, USA with automatic sampler Perkin-Elmer AS 93plus, Perkin-Elmer, USA, in three replicates, under following conditions: nebulizer gas flow rate 0.9 l/min, ICP-RF power 1000 W, lens voltage 7.75 V, analog stage voltage -1887 V, pulse stage voltage 1100 V, discriminator threshold 65 V and AC rod offset -2.9 V. Series of calibration solutions were prepared to determine the mass concentration of dissolved Ti using standard Perkin-Elmer Pure Plus Multi-Elemental Calibration Standard 5, Perkin-Elmer, USA.

The amount of Ti released for each specimen (µg/cm^2) was calculated from the data of the mass concentration in test solutions, blank test and surface area using formula: the amount of released Ti (µg/cm^2) = volume of test solution (l) · (mass concentration of Ti in test solution (µg/l) - mean mass concentration of Ti in blank test with three flasks (µg/l)) / surface area of specimen (cm^2). The quantity of dissolved Ti is considered zero at the Ti concentration below that of the blank test. The mean quantity and standard deviation were calculated for the three flasks.

Surface analysis

Specimen surface analysis was performed by means of scanning electron microscope (SEM) type VEGA TS5136LS, Tescan, Czech Republic, using SE and BSE detectors at a magnification of 50 and 200 times with electron beam accelsnopa od 20 kV. Kemijska mikroanaliza površine obavljena je energijskom disperzivnom spektroskopijom (EDS) koristeći se detektorom EDS (Oxford Instruments, Ujedinjeno Kraljevstvo). Za obradu EDS-podataka korišten je računalni program Inca (Oxford Instruments, Ujedinjeno Kraljevstvo).

Statistička analiza

Statistička analiza obavljena je računalnim paketom Statistica (Dell Software, SAD). Srednje vrijednosti i standardne devijacije količine otpuštenih iona titanija izračunate su osnovnim statističkim metodama. Za procjenu razlike u količini otpuštenih iona titanija između tri ispitivana materijala i četiri različite otopine korišteni su analiza varijance (ANOVA) i Newman-Keulsov test. Utjecaj vrste ispitivane otopine i vrste materijala na količinu otpuštenih iona titanija iz uzorka određivana je multiplom regresijskom analizom i generalnim regresijskim modelom. Razina značajnosti postavljena je na $p < 0,05$.

Rezultati

Količina otpuštenih iona titanija (Ti)

Kod ispitivanih materijala Ti-1Mg i Ti-2Mg nije bilo značajne razlike u količini otpuštenih iona titanija među ispitivanim otopinama. U HBSS-u je ispitivanje Ti-1Mg i Ti-2Mg pokazalo rezultat od 0,32 ± 0,05 µg/cm^2, odnosno, 0,32 ± 0,07 µg/cm^2. Značajna razlika mogla se uočiti u AS-u, u AS-pH4 i u AS-u s fluorom (F): 0,02 ± 0,03 µg/cm^2 nasuprot 0,04 ± 0,05 µg/cm^2, odnosno, 0,09 ± 0,13 µg/cm^2 nasuprot 0,00 ± 0,00 µg/cm^2, odnosno, 0,67 ± 0,01 µg/cm^2 nasuprot 0,48 ± 0,09 µg/cm^2. Ipak, uočljiva je bila razlika u količini otpuštenih iona titanija u AS-u s fluorom i HBSS-u. Kod CP Ti 4, AS i HBSS pokazali su sličan korozijski učinak te je količina otpuštenih iona titanija iznosila 0,93 ± 0,01 µg/cm^2. Samo malo veća količina otpuštenih iona titanija uočena je pri pH 4 (1,63 ± 0,01 µg/cm^2). Otapanje iona titanija poraslo je više od 11 puta u prisutnosti iona fluora i iznosilo je 10,80 ± 0,01 µg/cm^2. Slika 1. prikazuje usporedbu količine otpuštenih iona titanija između tri ispitivana materijala u četiri različite vrste otopina.

Analiza varijance (ANOVA) (tablica 1.) pokazala je statistički značajnu razliku u količini otpuštenih iona titanija između ispitivanih otopina u tri ispitivana materijala (Ti-1Mg: p = 0,002574; Ti-2Mg: p = 0,004284; CP Ti 4: p = 0,000000). Newman-Keulsov test otkrio je da kod kompozita Ti-1Mg postoji statistički značajna razlika u količini otpuštenih iona titanija između AS-a s fluorom i svih ostalih ispitivanih otopina (AS-pH 4 nasuprot AS s F p = 0,002792). Slično je i kod kompozita Ti-2Mg – zabilježena je statistički značajna razlika između AS-a i AS-a s fluorom (p =

eration voltage of 20 kV. Chemical microanalysis of the surface was performed by energy-dispersive spectroscopy (EDS) using the EDS detector, Oxford Instruments, UK. Inca software, Oxford Instruments, UK, was used to process the EDS data.

Statistical analysis

Statistical evaluation was performed by Statistica, Dell Software, USA, software package. Mean values and standard deviations for the amount of released Ti were calculated by basic statistic method. For the assessment of the differences in the amount of dissolved Ti among three materials tested and four different solutions, the analysis of variance (ANOVA) and Newman-Keuls test were used. The influence of the test solution and type of material on the amount of Ti released from the material was determined by multiple regression analysis and General regression model. A $p<0.05$ was taken to indicate statistical significance.

Results

Amount of released titanium ions (Ti)

In the case of experimental Ti-1Mg and Ti-2Mg, there was no significant difference in the amount of the released Ti ions among the test solutions. In HBSS Ti-1Mg and Ti-2Mg, the tests showed the result of 0.32 ± 0.05 µg/cm^2 and 0.32 ± 0.07 µg/cm^2, respectively. Significant difference can be observed in AS, AS-pH 4 and AS with F: 0.02 ± 0.03 µg/cm^2 vs. 0.04 ± 0.05 µg/cm^2, 0.09 ± 0.13 µg/cm^2 vs. 0.00 ± 0.00 µg/cm^2 and 0.67 ± 0.01 µg/cm^2 vs. 0.48 ± 0.09 µg/cm^2, respectively. However, the difference between the amount of dissolved Ti in AS with F and HBSS was evident. In the case of CP Ti 4, AS and HBSS exhibited similar corrosive ability and the amount of released Ti reached 0.93 ± 0.01 µg/cm^2. Only a slightly higher Ti releasing was observed at pH 4 (1.63 ± 0.01 µg/cm^2). The dissolving of Ti increased more than 11 times in the presence of F ions and the amount reached 10.80 ± 0.01 µg/cm^2. Figure 1 demonstrates the comparison of the amount of the released Ti among three tested materials in four different solutions.

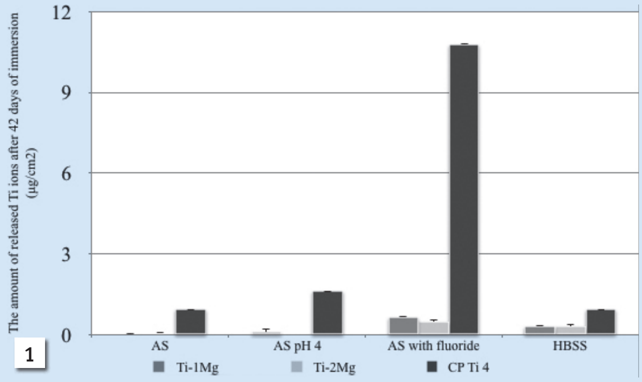
Analysis of variance (ANOVA) (Table 1) showed statistically significant difference in the amount of released Ti among test solutions for three tested materials (Ti-1Mg: p=0.002574; Ti-2Mg: p=0.004284; CP Ti 4: p=0.000000). Newman-Keuls test revealed that in the case of Ti-1Mg there was statistically significant difference in the amount of released Ti between AS with F and all other solutions (AS pH 4 vs. AS with fluoride p=0.002792). Similarly, in the case of Ti-2Mg there was statistically significant difference between AS and AS with F (p=0.001590). The results of multiple regres-

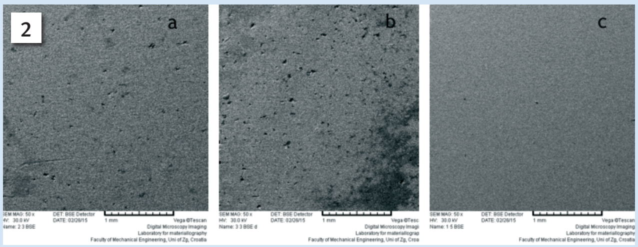
Tablica 1. Rezultati testiranja ANOVA-om za količinu otpuštenih iona titanija (Ti) iz ispitivanog materijala (Ti-1Mg, Ti-2Mg i CP Ti 4) u različitim otopinama

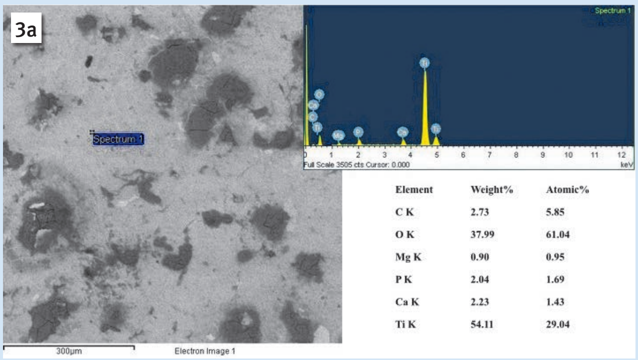
Table 1 Results of ANOVA testing for the amount of released Ti from tested materials (Ti-1Mg, Ti-2Mg, CP Ti 4) in different solutions.

Materijal • Material	SS učinak • SS Effect	df učinak • df Effect	MS učinak • MS Effect	SS pogreška • SS Error	df pogreška • df Error	MS pogreška • MS Error	F	p
Ti-1Mg	0.5	3	0.2	0.020	4	0.0050	34.4	0.002574*
Ti-2Mg	0.3	3	0.1	0.020	4	0.0040	26.3	0.004284*
CP Ti 4	209.93	3	69.98	0.00	8	0.00	699756.75	0.000000*

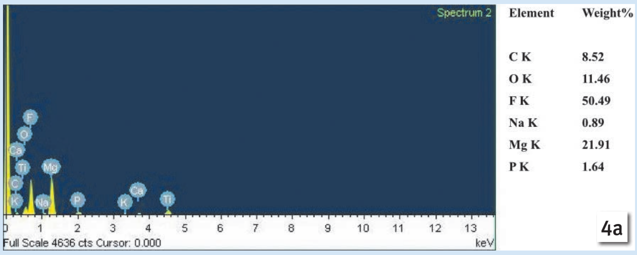
* statistički značajna razlika pri $p < 0,05$ • significantly different at $p<0.05$



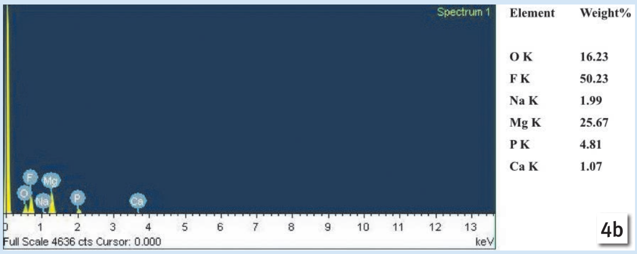




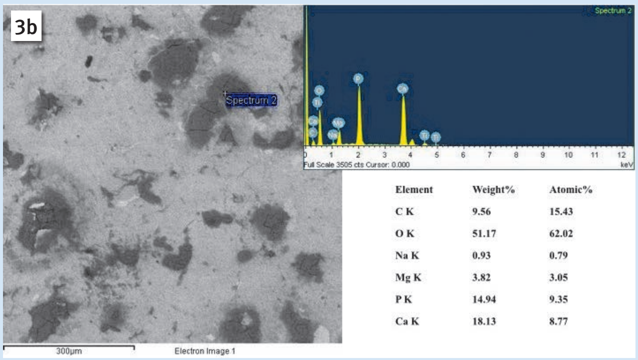
Element	Weight%	Atomic%
C K	2.73	5.85
O K	37.99	61.04
Mg K	0.90	0.95
P K	2.04	1.69
Ca K	2.23	1.43
Ti K	54.11	29.04



Element	Weight%
C K	8.52
O K	11.46
F K	50.49
Na K	0.89
Mg K	21.91
P K	1.64



Element	Weight%
O K	16.23
F K	50.23
Na K	1.99
Mg K	25.67
P K	4.81
Ca K	1.07



Element	Weight%	Atomic%
C K	9.56	15.43
O K	51.17	62.02
Na K	0.93	0.79
Mg K	3.82	3.05
P K	14.94	9.35
Ca K	18.13	8.77

Slika 1. Usporedba količine otpuštenih iona titanija (Ti) između tri ispitivana materijala u četiri različite ispitivane otopine: AS – umjetna slina; AS pH-4 – umjetna slina pH 4; AS i F – umjetna slina s fluorom; HBSS – Hankova otopina nakon 42 dana uranjanja.

Figure 1 Comparison of the amount of the released Ti among three tested materials in four different test solutions: AS - artificial saliva; AS pH-4 - artificial saliva-pH 4; AS with F - artificial saliva with fluoride; HBSS - Hank's balanced salt solution after 42 days of immersion

Slika 2. SEM-mikrosnimke ispitivanih (a) Ti-1Mg, (b) Ti-2Mg i (c) CP Ti 4 na povećanju od 50 puta koristeći se BSE-detektorom nakon 42 dana uranjanja u umjetnoj slini s fluorom

Figure 2 SEM micrographs of test (a) Ti-1Mg, (b) Ti-2Mg and (c) control CP Ti 4 at magnification of 50 times using BSE detector after 42 day immersion in artificial saliva with fluorides.

Slika 3. Mikrosnimke i EDS-analiza korozijskih produkata na površini uzorka sadržavaju većinom a) Ti i O, b) Ca, P i O, nakon 42 dana uranjanja u HBSS.

Figure 3 Micrographs and EDS analysis of corrosion products on the surface of test specimen containing a) Ti and O, b) Ca, P and O mainly, after 42 day immersion in HBSS.

Slika 4. EDS-analiza korozijskih produkata na ispitivanom uzorku a) Ti-1Mg i b) Ti-2Mg nakon 42 dana uranjanja u umjetnoj slini s fluorom sadržavaju većinom atome magnezija i fluora

Figure 4 EDS analysis of corrosion products on the surface of test a) Ti-1Mg and b) Ti-2Mg specimen after 42 day immersion in the artificial saliva with fluorides, containing Mg and F atoms mainly.

Tablica 2. Rezultati multiple regresijske analize za korelaciju između prediktornih varijabli i količine otpuštenih iona titanija (Ti)

Table 2 Results of multiple regression analysis testing for correlation between predictor variables and the amount of released Ti.

Prediktorska varijabla • Predictor variable	Statistički parametar • Statistical parameter	
	Beta	p
Otopina • Solution	0.67	0.006078*
Materijal • Alloy	0.14	0.496139
	R=0.68; p<0.01766*	

* statistički značajna razlika pri p < 0,05 • significantly different at p<0.05

0,001590). Rezultati multiple regresijske analize (tablica 2.) pokazali su dobru, statistički značajnu korelaciju (R = 0,68; p < 0,01766) između prediktornih varijabli (ispitivane otopine, materijal) i količine otpuštenih iona titanija. Prema beta-koeficijentu, samo je vrsta ispitivane otopine imala statistički značajan utjecaj na otpuštanje iona titanija (p = 0,006078).

Analiza površine

SEM-mikrosnimke kompozita Ti-1Mg, Ti-2Mg i CP Ti 4 učinjene detektorom BSE, kao što je prikazano na slici 2., pokazale su očuvanu dvofaznu površinsku strukturu ispitivanih uzoraka nakon 42 dana uranjanja, s diskretno formiranim produktima korozije. EDS-analiza korozijskih produkata na površini uzoraka uronjenih u HBSS (slika 3.) pokazala je da se oni sastoje većinom od atoma titanija i kisika (Ti i O) ili kalcija, fosfora i kisika (Ca, P i O). S druge strane, produkti nastali na uzorcima uronjenim u AS s fluorom (slika 4.) pokazali su nakon EDS-analize prisutnost magnezija (Mg) i fluora (F).

Rasprava

Komercijalno čisti titanij koji se uvelike koristi u kliničkoj dentalnoj medicini pokazuje vrlo slabu korozijsku otpornost u uvjetima s niskim pH i u prisutnosti fluora. Ipak, samo dodavanje fluora umjetnoj slini povećava količinu otpuštenih iona titanija za 10 puta, što je u skladu s pretpostavkom o fluoru kao o vrlo agresivnom i reaktivnom halogenom elementu koji može razoriti zaštitni dioksidni sloj. Slično su u svojem istraživanju *in vitro* pokazali Milošev i suradnici – dobili su visoku stopu korozije titanija u prisutnosti fluora nakon 32 dana uranjanja (7). Istraživanje Sartorija i suradnika (32), provedeno na dentalnim implantatima izrađenima od CP Ti 4 tretiranima s fluorom, koristeći se samo metodama SEM i EDS, pokazalo je da nema znakova korozije na površini uzoraka. Suprotno tome, ovo istraživanje pokazalo je da, iako nema znakova korozije ako se promatra SEM-om, postoji velika količina otpuštenih iona titanija (Ti) s površine CP Ti 4 u prisutnosti fluora. Na temelju toga može se zaključiti da promatranje SEM-om nije dostatno za procjenu korozijskog ponašanja, pogotovo ako je korozija prisutna u generaliziranom obliku bez pojave jamica i pukotina.

U usporedbi s CP Ti 4, eksperimentalni Ti-1Mg i Ti-2Mg pokazuju sličnu tendenciju s obzirom na količinu otopljenih iona titanija (Ti) između četiri različite ispitivane otopine. Najveća vrijednost i statistički značajna razlika uočena je u AS-u s fluorom. Dodavanje novih komponenti titanijskoj osnovi može biti strategija kako poboljšati korozijsko ponašanje novoga materijala i postići bolju biokompatibilnost. Rosalbino i suradnici ustanovili su u elektrokemijskom istraživanju bolju korozijsku otpornost ispitivanog materijala proizvedenog dodavanjem plemenitih metala titaniju (33). Fojt i suradnici su, u istraživanju s legurom Ti-39Nb, pokazali da proces metalurgije praha i posljedična poroznost mogu biti razlog za bolju korozijsku otpornost takvog materijala (34).

Osim toga, magnezij i mikrogalvanski korozijski učinak mogu rezultirati boljim korozijskim ponašanjem ispitivanog materijala iz ovog istraživanja. Galvanska korozija nastaje kada se dvije različite kovine nalaze u fizičkom i električnom kontaktu u vodenoj otopini. Dvofazna struktura materijala

sion analysis (Table 2) showed good, statistically significant correlation (R=0.68; p<0.01766) between predictor variables (test solution, material) and the amount of released Ti. According to beta coefficients, only the type of the test solution had statistically significant influence on the Ti dissolution (p=0.006078).

Surface analysis

SEM micrographs with BSE detector of Ti-1Mg, Ti-2Mg and CP Ti 4, as shown in Figure 2, demonstrated preserved heterogeneous dual phase surface structure of test specimens after 42 day immersion with discreetly formed corrosion products. EDS analysis of corrosion precipitates on the surface of the specimen immersed in HBSS (Figure 3) showed that they were mainly composed of atoms of Ti and O or Ca, P and O. On the other hand, precipitates formed on test specimens immersed in the AS with fluorides (Figure 4) using EDS analysis, demonstrated the presence of Mg and F.

Discussion

Commercially pure titanium, widely used in clinical dental medicine, shows very poor corrosion resistance in the condition of low pH and the presence of F. However, only the addition of F in AS increases the amount of released Ti ions for one order of magnitude that is consistent with a presumption of F as a very aggressive and reactive halogen that can destroy the protective dioxide film. Similarly, Milošev et al. in an *in vitro* study demonstrated a high rate of titanium corrosion in the presence of F after 32 days of immersion (7). Study of Sartori et al. (32), performed on dental implants made from CP Ti 4, treated with F and used only SEM and EDS method, showed that there was no evidence of corrosion on the specimens surfaces. In contrast, the present study demonstrated that there were no signs of corrosion observed using SEM analysis, there was a high amount of released Ti from the surface of CP Ti 4 in the presence of fluoride. This can lead to the conclusion that SEM observation is not a sufficient method to assess the corrosion behavior especially if the corrosion is presented in a generalized form without the appearance of pits or crevices.

When compared to CP Ti 4, the experimental Ti-1Mg and Ti-2Mg demonstrate similar tendency in respect of the amount of dissolved Ti ions among four different test solutions. The highest value and statistically significant difference were observed in AS with F. The addition of new components to titanium matrix can be the strategy to improve the corrosion behavior of a new material and achieve better biocompatibility. Rosalbino et al., in an electrochemical study, demonstrated better corrosion resistance of studied material produced by adding noble metals to titanium (33). Fojt et al., in the study with Ti-39Nb alloy, reported that the process of powder metallurgy and consequent porosity could be the reason for better corrosion resistance of such materials (34).

Moreover, the presence of magnesium and micro-galvanic corrosion effect may lead to improved corrosion behavior of tested material in the present study. Galvanic corrosion occurs when two dissimilar metals are in physical and electrical contact in an aqueous solution. The dual phase structure of Ti-Mg material and direct inter-metallic contact between

Ti-Mg i izravan interkovinski kontakt između titanijske i magnezijske komponente mogu uzrokovati da elektronegativnija kovina kao anoda, što je u ovom slučaju magnezij, štiti drugu (titanij) kao katodu od korozije, tj. otapanja. Osim toga, magnezij pasivizira stvarajući netopljivi magnezijev hidroksid ($Mg(OH)_2$) koji se u fluoridiranim otopinama pretvara u magnezijev fluorid (MgF_2), također visoko netopljiv sloj koji štiti površinu od daljnje korozije. U HBSS-u, visoka koncentracija iona klora razara pasivni sloj $Mg(OH)_2$ stvarajući magnezijev klorid ($MgCl_2$) koji je topljiv. Posljedica je povećano otapanje magnezija. Takva reakcija može potaknuti pasivaciju titanija stvarajući titanijev oksid (TiO_2) i taložeći sloj hidroksilapatita (HA). Slično su ustanovili Jung i suradnici, a to je poboljšano stvaranje i rast jezgri HA-kristala u prisutnosti magnezija (35). Isti mehanizam može potaknuti bioaktivnost eksperimentalnog materijala od titanij-magnezija.

No, za bolje razumijevanje kemijskih i bioloških svojstava, karakteristika površina i potencijalne bioaktivnosti potrebna su daljnja istraživanja.

Zaključak

Dobiveni su sljedeći zaključci:

1. Prikazani rezultati potvrđuju malu količinu otpuštenih iona titanija iz inovativnog eksperimentalnog materijala. Uočena je do 46 puta, odnosno 23 puta manja količina otpuštenih iona titanija (Ti) iz materijala Ti-1Mg, odnosno Ti-2Mg u usporedbi s čistim titanijem (CP Ti).
2. Korozijsko ponašanje uvelike ovisi o vrsti ispitivane otopine. Između ispitivanih otopina, AS s fluorom pokazuje najveći korozijski učinak na sva tri ispitivana materijala.
3. Sadržaj magnezija u rasponu od 1 i 2 masena udjela ne utječe značajno na korozijsko ponašanje dvaju ispitivanih eksperimentalnih materijala.

Iz gore spomenutoga može se zaključiti da kompoziti Ti-1Mg i Ti-2Mg imaju visoku korozijsku otpornost. Statički test uranjanja proveden kemijskim modelom (ispitivane otopine) koji oponaša biološke uvjete samo djelomično i može biti ograničenje u ovom istraživanju. Ipak, može se sugerirati šira upotreba ispitivanog materijala u dentalnoj medicini.

Sukob interesa

Autori nisu ni u kakvom sukobu interesa.

titanium and Mg components could cause the more electronegative metal as anode, which is in this case Mg, to protect the other one (titanium) as cathode from the corrosion, i.e., dissolving. Additionally, magnesium aggravates forming insoluble magnesium hydroxide ($Mg(OH)_2$), which in fluoridated solution turns into magnesium fluoride (MgF_2), also a highly insoluble layer that protects the surface from further corrosion. In HBSS, high concentration of chloride ions distorts the passive layer of $Mg(OH)_2$ by forming magnesium chloride ($MgCl_2$) which is soluble. The result is increased dissolution of Mg. Such a reaction could promote the passivation of titanium by forming a titanium-oxide (TiO_2) and deposing a hydroxyapatite (HA) layer. Similarly, Jung et al. reported improved nucleation and growth of HA crystals in the presence of Mg (35). The same mechanism may favor bioactivity in titanium-magnesium experimental material.

However, further research is needed for a better understanding of the chemical and biological properties, surface characteristics and potential bioactivity of this material.

Conclusion

The following conclusions were reached:

1. Reported results confirm low release of Ti from innovative experimental material. Up to 46- and 23- fold lower amount of dissolved Ti from Ti-1Mg and Ti-2Mg, respectively was observed when compared to control CP Ti.
2. The corrosion behavior is highly dependent on the type of test solution. Among the tested solutions, AS with F exhibited the highest corrosion effect on all three materials tested.
3. Mg content in the range of 1 and 2 mass% had no significant influence on corrosion behavior of the two tested experimental materials.

From the above mentioned, one can conclude that the Ti-1Mg and Ti-2Mg materials have high corrosion resistance. The static immersion test performed by using chemical models (test solutions) which can mimic the real biological environment only partially, could be a limitation of the present study. However, possible wider application of tested materials in dental medicine is suggested.

Conflict of interest

The authors deny any conflict of interest

Abstract

The innovative titanium-magnesium composite (Ti-Mg) was produced by powder metallurgy (P/M) method and is characterized in terms of corrosion behavior. **Material and methods:** Two groups of experimental material, 1 mass% (Ti-1Mg) and 2 mass% (Ti-2Mg) of magnesium in titanium matrix, were tested and compared to commercially pure titanium (CP Ti). Immersion test and chemical analysis of four solutions: artificial saliva; artificial saliva pH 4; artificial saliva with fluoride and Hank balanced salt solution were performed after 42 days of immersion, using inductively coupled plasma mass spectrometry (ICP-MS) to detect the amount of released titanium ions (Ti). SEM and EDS analysis were used for surface characterization. **Results:** The difference between the results from different test solutions was assessed by ANOVA and Newman-Keuls test at p<0.05. The influence of predictor variables was found by multiple regression analysis. The results of the present study revealed a low corrosion rate of titanium from the experimental Ti-Mg group. Up to 46 and 23 times lower dissolution of Ti from Ti-1Mg and Ti-2Mg, respectively was observed compared to the control group. Among the tested solutions, artificial saliva with fluorides exhibited the highest corrosion effect on all specimens tested. SEM micrographs showed preserved dual phase surface structure and EDS analysis suggested a favorable surface bioactivity. **Conclusion:** In conclusion, Ti-Mg produced by P/M as a material with better corrosion properties when compared to CP Ti is suggested.

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Key words
Titanium; Magnesium; Corrosion; Immersion; Saliva, Artificial; Ion Exchange

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