Janko Ferčec[1], Rebeka Rudolf[1,2]
[1] Fakulteta za strojništvo, Univerza Maribor / Faculty of Mechanical Engineering, University of Maribor, Smetanova 17, 2000 Maribor, Slovenija / Slovenia
[2]Zlatarna Celje d.d., Kersnikova 19, 3000 Celje, Slovenija / Slovenia

Nikelj-titanova zlitina za ortodontsko žico za loke: izdelava, funkcionalne lastnosti in biokompatibilnost

Nickel–titanium alloy for orthodontic arch-wire: Manufacture, functional properties and biocompatibility

Izvleček

Nikelj-titanove zlitine so postale zelo uporabljan material v medicinskih aplikacijah, še posebej za fiksne ortodontske aparate pri zdravljenju neporavnanih zob. Razlog je v tem, da imajo ti materiali funkcionalne lastnosti in da so biokompatibilni. Ta prispevek se osredotoča na pojasnitev procesa izdelave zlitin z oblikovnim spominom, na ugotavljanje njihovih funkcionalnih lastnosti ter na ugotovitev stopnje biokompatibilnosti. Pri ugotavljanju funkcionalnih lastnosti smo merili temperaturo faze, pri kateri ima zlitina z oblikovnim spominom popolno avstenitno mikrostrukturo. Nato smo ugotavljali mehanske lastnosti z enoosnim nateznim preskusom. V delu biokompatibilnosti smo prikazali postopek, s katerim je mogoče oceniti biološko ustreznost ortodontskih žic.

Vsi ti rezultati nam posredno pomagajo pojasniti postopek in potek ortodontskega zdravljenja, še posebej za primer uporabe različnih komercialno dostopnih Ni-Ti ortodontskih žic.

Ključne besede: zlitine z oblikovnim spominom, izdelava, funkcionalne lastnosti, biokompatibilnost

Abstract

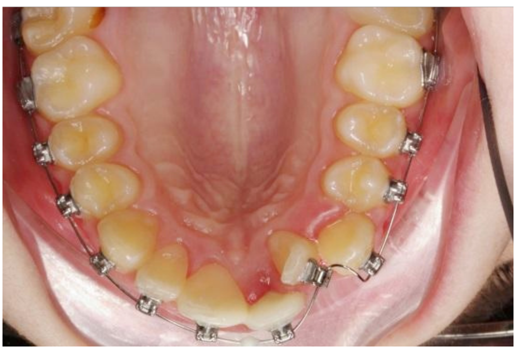
Nickel-titanium alloys have become a widely used material in medical applications, especially in orthodontic appliances for treatment of protruding teeth. The reason for this is that this material has specific functional properties and is biocompatible. This paper concentrates on the clarification of the process of manufacture of shape memory alloys and determination of the functional properties and biocompatibility of different orthodontic wires from this alloy. When establishing the functional features, we first determined the temperature of the phase in which the shape memory alloy has completely austenitic microstructure. Then we determined the mechanical properties with a uni-axial tension test.

This helps in the understanding of orthodontic treatment using different commercially available orthodontic arch-wires from nickel-titanium alloy. In the section on biocompatibility the process of determining the biocompatibility of orthodontic wires is shown.

Key words: shape memory alloy, manufacture, functional properties, biocompatibility

1 Uvod

Žica iz zlitine z oblikovnim spominom se uporablja za številne pripomočke v medicini, vključno z vodilnimi žicami, katetri, žilnimi opornicami (stenti), filtri, iglami, vodilnimi čepi, endodontskimi pilami in ortodontski pripomočki. Pri ortodontski popravi naprej štrlečih zob se uporablja tehnika nežnega in stalnega pritiska na zobe. Sila, ki deluje na zob, ustvarja napetosti, ki delujejo najprej na zob, potem pa se te prenašajo na periodontalni ligament. Te napetosti nato povzročijo spremembe v prekrvavitvi periodontalnega ligamenta, kar vodi v preoblikovanje čeljusti. Istočasno se uporablja za pomik zoba v pravilno lego v ustni votlini. Za učinkovito delovanje sil na več zob hkrati se uporabljajo fiksni ortodontski pripomočki (slika 1). Ti ortodontski pripomočki so sestavljeni iz konzol, ki so pritrjene na krono zoba tako, da so prilepljene ali pritrjene na zob s posebnimi trakovi. Ko ortodont namesti konzole na vsak zob zgornje ali spodnje čeljusti, se uporabi ortodontska žica. Žica za loke, ki se vstavi v utore na konzolah, deluje s silo na zob, s čemer počasi pomika zob med zdravljenjem [1-3].



Slika 1. Ortodontski pripomoček iz nikelj-titanove ortodontske žice z oblikovnim spominom

Figure 1. Orthodontic appliance by SMA NiTi orthodontic wire

1 Introduction

Shape Memory Alloy (SMA) wire is used in a variety of medical device applications including guide wires, catheters, stents, filters, needles, guide pins, endodontic files and orthodontic appliances. In the process of the orthodontic treatment of protruding teeth the technique of a gentle and continuous force is used on the teeth. The force exerted on the tooth to create stresses acts first on the tooth and then it is transferred to the periodontal ligament. These stresses subsequently cause a change in the blood supply to the periodontal ligament, leading to the transformation of the jaw. At the same time, it is used to move teeth into their correct position in the oral cavity. For efficient operation of forces on several teeth at the same time fixed orthodontic appliances are used (Figure 1). These orthodontic appliances consist of brackets which are attached to the crown of the tooth so that they are glued or fastened to the teeth with special bands. When the orthodontist has placed the bracket on each tooth on the upper or lower jaw, the orthodontic wire is then introduced. The arch-wire, which is inserted into the slots in the brackets, causes force on the tooth and, consequently, the movement during the course of treatment [1-3].

The desirable properties of orthodontic wires are, mainly, the following: Good biocompatibility, good spring-back, good range, and tough and low fraction. It's important also that the formability is resilient, so that the orthodontist may deform it into loops or a band fused onto a clasp, and must have the ability to return to its original shape. The wire must also be the most aesthetic, so that it does not disturb the looks of the human mouth. An ideal arch-wire with ideal properties does not exist.

Želene lastnosti ortodontskih žic so predvsem naslednje: dobra biokompatibilnost, dobra zaostala elastičnost, dobra povratna deformacija, žilavost in majhna lomljivost. Pomembno je tudi, da se žica lahko prožno oblikuje, ko jo ortodont deformira v zanko ali ko trak pritali na spono ter se potem vrne v prvotno obliko. Žica mora imeti tudi čim bolj estetski videz, tako da ne moti pogleda v človeška usta. Idealna žica za loke z idealnimi lastnostmi ne obstaja.

V zadnjih letih se je zlitina NiTi z oblikovnim spominom začela uporabljati v začetni fazi ortodontskega zdravljenja. Ta material se je uveljavil v ortodontski praksi zaradi svojih funkcionalnih mehanskih lastnosti. Prvič ga je leta 1975 uporabil v ortodontske namene dr. Andreasen z Univerze Iowa [4].

Vzrok, zakaj se je zlitina NiTi z oblikovnim spominom začela uporabljati v ortodontski praksi, je njena dobra biokompatibilnost in ker ima specifično mehansko lastnost, superelastičnost, ki jo imajo zlitine z oblikovnim spominom. Pomembna funkcionalna lastnost zlitin z oblikovnim spominom, ki se uporabljajo za ortodontske žice, je majhen modul elastičnosti, kar ustvarja majhno silo na zobe in veliko povratno deformacijo, dodatno pa vzdržuje konstantno silo med celotnim zdravljenjem. Te lastnosti so pomembne za ortodontsko popravo zob, kar uspešno izboljša superelastičnost zlitine NiTi z oblikovnim spominom [5].

V tem prispevku je predstavljena izdelava zlitine z oblikovnim spominom skupaj z rezultati temperaturnih meritev fazne premene, ki so bile narejene z diferencialno vrstično kalorimetrijo (DSC). Meritve modula elastičnosti in značilnega raztezka trgovsko dosegljivih ortodontskih žic za loke iz zlitin NiTi z oblikovnim spominom so prikazane grafično. Na koncu

In recent years SMA NiTi came into use in the initial stage of the orthodontic treatment. This material has been established in orthodontic practice because of its functional mechanical properties. For orthodontic purposes it was first introduced in 1975 by Dr. Andreasen of the University of Iowa [4].

The reason why the SMA NiTi was introduced into orthodontic practice is that it has good biocompatibility and has a specific mechanical property (super elasticity), which SMAs possess. An important feature of the functional SMA used for the orthodontic wire is the low modulus of elasticity, which creates a small force on the teeth and large recoverable strain (range), which, in turn, creates a continuous duration of force during orthodontic treatment. These properties are important in the process of orthodontic treatment and this improves the superelasticity of SMA NiTi successfully [5].

In the present work is presented SMA manufacture, together with the results of measuring the temperature of the phase transition with Differential Scanning Calorimetry (DSC). On the graphs we showed measurements of the modulus of elasticity and characteristic elongation on commercially available SMA NiTi orthodontic arch-wire. At the end of the paper we showed the test of the biocompatibility of orthodontic wire.

2 Manufacture of Ni-Ti alloy

The chemical composition of the SMA NiTi is important because the properties are very sensitive to the inital chemistry. NiTi alloys have almost equiatomic composition. The chemical composition is very important in SMA because it has a decisive influence on the phase transitions temperature. The equiatomic composition (50 at.% Ni je prikazan tudi biokompatibilnostni preskus ortodontske žice.

2 Izdelava zlitine NiTi

Kemična sestava zlitine NiTi z oblikovnim spominom je pomembna, ker so lastnosti zelo občutljive na začetno kemično sestavo. Zlitine NiTi imajo skoraj ekviatomsko sestavo. Kemična sestava je zelo pomembna pri zlitinah z oblikovnim spominom, ker odločilno vpliva na temperaturo fazne premene. Zlitina z ekviatomsko sestavo (50 at.% Ni in 50 at. % Ti) ima najvišjo temperaturo A_f 120 °C. S povečevanjem deleža nikljevih atomov se temperatura premene znižuje. Pri 51 at. % je temperatura premene A_f – 40 °C [6].

Izdelava zlitine NiTi je zapletena. Slika 2 prikazuje shematično zaporedje postopkov [5,7]:

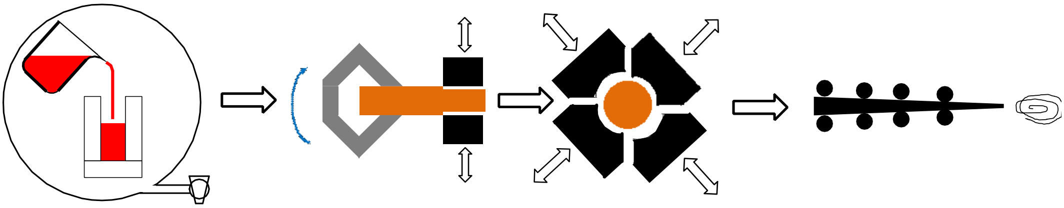
1. Taljenje/ulivanje v vakuumu: nikelj-aluminijeve zlitine se pogosto izdelujejo z indukcijskim taljenjem v vakuumu v grafitnem talilniku, ker je staljena zlitina NiTi zelo reaktivna, če je titana blizu petdeset odstotkov. Za indukcijsko taljenje v vakuumu se najraje uporabljajo talilniki iz grafita ali CaO, ker drugi materiali onesnažijo staljeni NiTi s kisikom. Surovine se določijo, preden se zlitina stali z vakuumskim obločnim pretaljevanjem. Ta postopek se uporablja, ker omogoča najboljšo možno homogenost in čistost zlitine. Dvojno vakuumsko taljenje zagotavlja kakovost in doseganje mehanskih lastnosti zlitine.
2. Ingoti se vroče preoblikujejo s stiskalnim kovanjem.
3. Sledi rotacijsko krčilno kovanje do različnih oblik. Optimalna temperatura za vroče preoblikovanje je okoli 800 °C. Pri tej temperaturi je zlitina dobro preoblikovalna, niti se ne pojavlja

and 50 at.% Ti) exhibits the maximum A_f temperature 120 °C. By increasing the value of Ni atomic percentage, the transformation temperature decreases. For 51 at.% nickel it is A_f -40 °C [6].

Production of nickel-titanium is a complex process. Figure 2 shows a schematic view of the proccesses that occur after the following [5, 7]:

1. Vacuum melting/casting: Manufacture of nickel-titanium alloys is often done by Vacuum Induction Melting (VIM) in a graphite crucible. The reason we used VIM is because the molten state of NiTi is very reactive if Ti is close to fifty percent. The graphite or calcia (CaO) crucible is preferred for VIM because the others contaminate the molten NiTi with oxygen. The raw materials are formulated before the alloy is melted by Vacuum Arc Remelting (VAR). This VAR process takes place in order to achieve the best possible homogeneity and purity of the alloy. The double vacuum melting process ensures quality and maintains the mechanical properties of the alloy.
2. The ingots are hot worked with press forging.
3. Follow rotary swaging to different shapes. The optimal temperature for hot working appears to be around 800 °C. At this temperature the alloy is easily workable and there is not too severe oxidation of the surface.
4. Following cold worked on sizes (rolling) according to the product (rod, wire). Cold working of NiTi is complex because the alloy work-hardens rapidly.

The procedure (Figure 3) of hot and cold treatments, followed by the rolling procedure, obtained a tapered shape, followed by the procedure of annealing the wire or rod into a coiled state. Due to work-hardness it requires multiple reductions



1. taljenje/litje v vakuumu 2. stiskalno kovanje 3. rotacijsko krčilno kovanje 4. vlečenje palic/žice
1. Vacuum melting/casting 2. Press forging 3. Rotary swaging 4. Rod/wire rolling

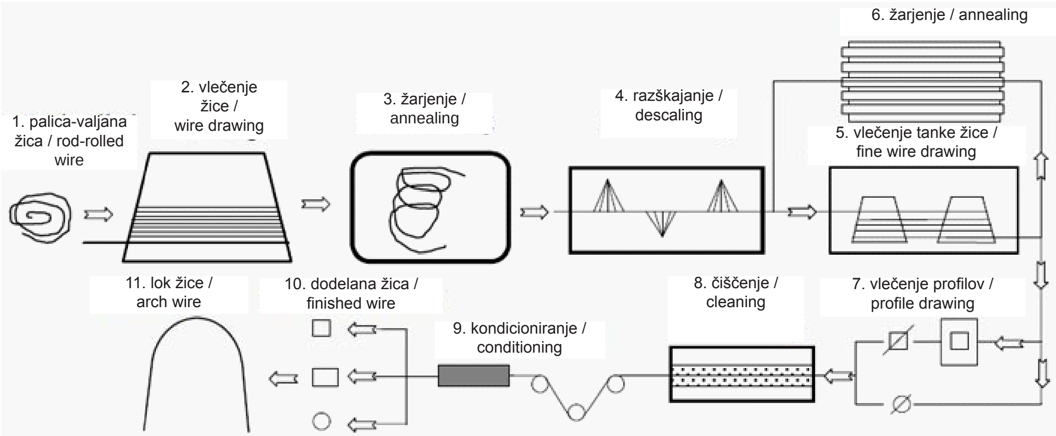
Slika 2. *Shematičen prikaz izdelavnega postopka za zlitino NiTi*
Figure 2. Schematic representation of the manufacturing process of NiTi alloy

močna oksidacija površine.

4. Sledi hladno preoblikovanje (*valjanje*) v končne oblike (palice, žica). Hladno preoblikovanje zlitine NiTi je zapleteno, ker se zlitina hitro preoblikovalno utrjuje.

Postopek (slika 3) je sestavljen iz vroče in hladne predelave, sledi valjanje žice, ki se navija na koničnem navijalniku, nato žarjenje debele ali tanke žice v zvitkih. Zaradi preoblikovalnega utrjevanja je pri valjanju potrebna redukcija v več stopnjah in pogosto medfazno žarjenje pri 600 – 800 °C. Sledi razškajanje in nato vlečenje tanke žice ter ponovno žarjenje. Po teh fazah predelave sledi izdelava končnih profilov (okroglo, kvadratno, pravokotno). Pravokotne žice se izdelujejo z vlečenjem okroglih žic. Zaradi velike povratne elastičnosti zlitine NiTi z oblikovnim spominom smo imeli nekaj težav pri vročem in hladnem preoblikovanju, ker je to zlitino težko oblikovati pri sobni temperaturi. Naslednji korak je bilo čiščenje žice, ki mu je sledilo učenje žice. Učenje je termomehanska obdelava, da se dosežejo optimalne lastnosti. Superelastični materiali NiTi se obdelujejo pri okoli 500 °C. Za zlitine z oblikovnim spominom je primerna temperatura v območju 350 – 400 °C. S strojno obdelavo se dobi končno obliko. Zlitina NiTi se lahko strojno obdeluje na standardne načine, kot je frezanje ali struženje, a je težava z obrabo orodja. Žice iz

and frequent inter-pass annealing at 600-800 °C. This is followed by the procedure of descaling, and then comes the process of fine drawing of wire and again annealing. After these phases follows the actual production profile (round, square, rectangular). The rectangular wires can be manufactured by drawing round wires. Due to the high spring-back of SMA NiTi we had some problems in hot and cold work as this alloy is difficult to form at an ambient temperature. The next step is then cleaning of the wire, followed by training of the wire. Training is thermo-mechanical treatment to achieve the optimized properties. Superelastic NiTi materials are heat treated in the vicinity of 500 °C. For shape memory alloys a suitable temperature is in the range between 350 °C and 450 °C. The machining is then made of the final profile. NiTi can be machined using conventional techniques such as milling, turning, but there are problems with tool wear. SMA NiTi wires we can be sheared and blanked quite effectively with proper tool design and maintenance. We can use this material successfully for abrasive processes such as grinding, sawing. This is important at the end of production for orthodontics; it is arch-wire [5, 7].



Slika 3. Shematičen prikaz izdelave končne NiTi-žice
Figure 3. Schematic representation of the production of finished NiTi wire

zlitine NiTi z oblikovnim spominom se lahko učinkovito režejo ali štancajo s primernim orodjem in njegovim vzdrževanjem. Lahko se tudi brusijo in žagajo. To je pomembno za izdelavo končnega ortodontskega izdelka, kar je žica za loke. [5,7].

3 Osnovni pojav

Zlitina NiTi kaže termoelastično martenzitno premeno. Ta premena povzroča pri zlitini ali oblikovni spomin ali superelastičnost.

3.1 Oblikovni spomin

Izraz oblikovni spomin izvira iz edinstvene sposobnosti zlitin da se 'spomnijo' predhodno določene oblike: celo po močni deformaciji za več odstotkov so se zlitine sposobne spontano vrniti v prvotno, predhodno določeno obliko pri določenih toplotnih razmerah. Za zlitino z oblikovnim spominom je značilno, da pri njej pri določeni temperaturi nastopi fazna premena. Te temperature prikazuje slika 4 in se jih lahko opiše na naslednji način: Temperatura Ms (začetek martenzitne premene) je

3 Basic phenomenon

NiTi alloy exhibits a thermoelastic martensitic transformation. This transformation is responsible for either shape memory or superelasticity being exhibited by the alloy.

3.1 Shape memory

The term shape memory stems from its unique ability to 'memorize' predetermined shape(s): Even after severe deformation of several percent (strain), they are capable of returning spontaneously to their original or parent, pre-deformed shape under certain thermal conditions. SMA is characterized with the characteristic temperatures of phase transformation. These temperatures are presented in Figure 4 and described as follows. Temperature Ms (martensite-start) is the temperature at which the austenite cools down and begins to transform to martensite. Temperature Mf (martensite-finish) is the temperature at which the material has a complete twinned martensite. Temperature As (austenite-start) is the temperature at which martensite begins to change into austenite. Temperature Af temperatura, pri kateri se avstenit ohladi in začne pretvarjati v martenzit. Temperatura Mt (konec martenzitne premene) je temperatura, pri kateri se je material v celoti pretvoril v martenzit. Temperatura As (začetek nastajanja avstenita) je temperatura, pri kateri se martenzit začenja spreminjati v avstenit. Temperatura Af (konec nastajanja avstenita) je temperatura, pri kateri je končana pretvorba v avstenit [6].

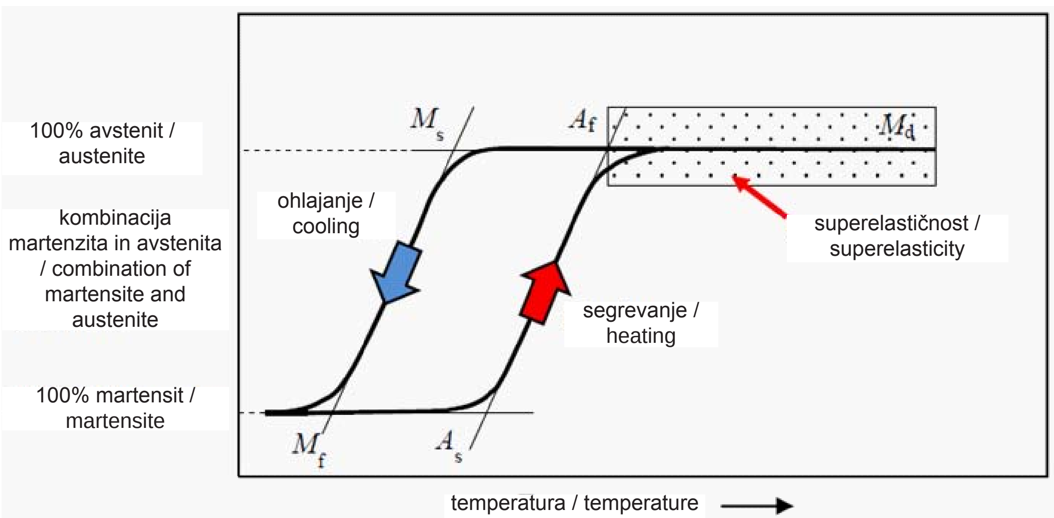
Pod temperaturo M_f se mikrostruktura zlitine z oblikovnim spominom pretvori iz avstenita s telesno centrirano kubično mrežo v martenzit z monoklinsko kristalno zgradbo (slika 5). Pod vplivom deformacije se zlitina z oblikovnim spominom pretvori v deformirani ali razdvojčeni martenzit. S segrevanjem se lahko zlitina z oblikovnim spominom pri temperaturi nad temperaturo A_f vrne v prvotno fazo.

Slika 5 Makroskopska in mikroskopska predstavitev pojava oblikovnega spomina

(austenite-finish) is the temperature at which the transformation in the austenite phase is completed [6].

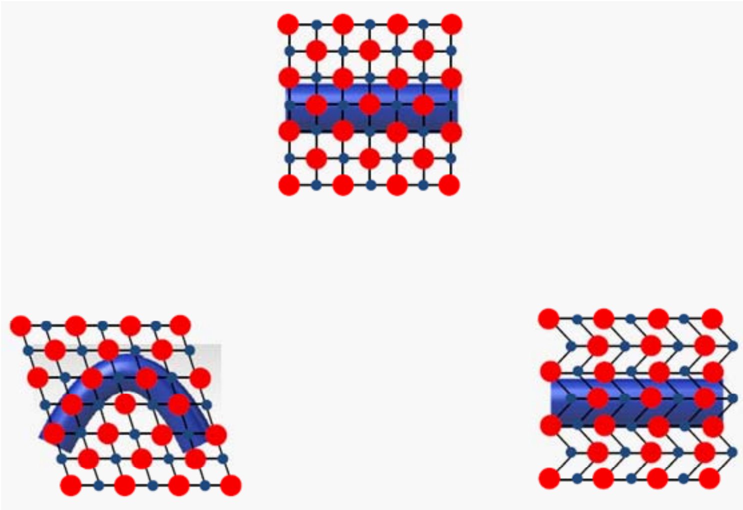
Under temperature Mf the structure of the SMA transforms from austenite with a body centered cubic (BCC) crystal structure to martensite with a monoclinic crystal structure (Figure 5). Under the influence of the deformation, the SMA deforms and transforms to deformed or detwinned martensite. By heating the SMA above the temperature Af it can be returned to the original (parent) phase.

The natural characteristic of materials with shape memory is to memorize their shape before pseudo-plastic deformation in the austenite state (one-way), but not the shape which has been obtained by the deformation. This can be learned with training. Training is cyclic thermo-mechanical treatment. In the case of a two-way memory formative gives two conditions:



Slika 4. Histereza martenzitne premene – zlitina z oblikovnim spominom se v temperaturnem območju od A_f do M_d obnaša superelastično

Figure 4. Hysteresis at martensitic transformation – SMA has, from the temperature A_f to M_d, superelasticity behaviour



Slika 5. Makroskopska in mikroskopska predstavitev pojava oblikovnega spomina

Figure 5. Macroscopic and microscopic presentation of shape memory effect

Naravna značilnost materialov z oblikovnim spominom je, da se spomnijo svoje oblike pred psevdoplastično deformacijo v avstenitno stanje (enosmerni spomin), ne pa oblike, ki je bila dosežena z deformacijo. To se lahko nauči z učenjem. Učenje je krožna termomehanska obdelava. Pri dvosmernem spominu je mikrostruktura, ki se pretvarja, lahko ali v martenzitnem stanju ali v avstenitnem stanju [6].

3.2 Superelastičnost

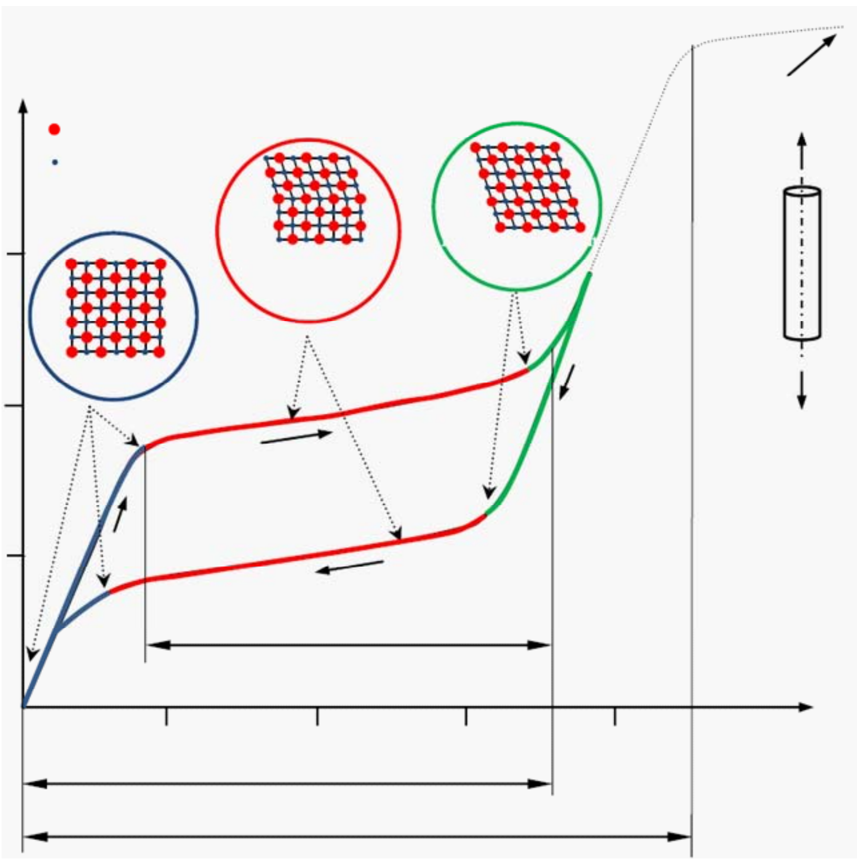
Superelastičnost je lastnost ali sposobnost materialov, ki so bili močno deformirani, za 6 – 10 %, da se po razbremenitvi vrnejo v prvotno stanje ali obliko [7]. Za superelatičnost je značilno, da nastopi, ko je material deformiran nad temperaturo A_f in zunanje napetosti povzročijo premeno osnovne avstenitne faze v martenzitno fazo (slika 6). Ta premena je brezdifuzijska premena v trdnem stanju iz kristalografsko višje urejene osnovne faze (telesno centrirani kubični avstenit) v kristalografsko nižje urejeno fazo (monoklinski martenzit) [8]. Premeno povzroči popačenje mreže

One in the martensitic state and the other in an austenitic state [6].

3.2 Superelasticity

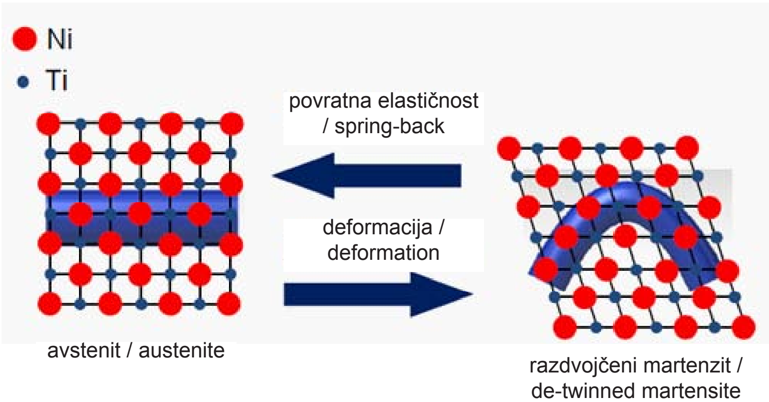
Superelasticity is the property or ability of materials to return a very high strain of ~ 6-10% in the original position or shape spontaneously upon unloading (Figure 7). Superelasticity is caused typically when a material is deformed above Af, where external applied stress induces the transformation of the parent austenite phase to a martensitic phase (Figure 6). This transformation takes place in the solid-solid diffusion-less phase transformation between the crystallographically more ordered parent phase (austenite (BCC)) and the crystallographically less ordered product phase (martensite (monoclinic)) [8]. Transformation caused the lattice distortion of the martensite from the austenite [9].The austenite is the stable phase under stress-free conditions. In the case of application of a critical stress, the austenite yields and starts transformation to the martensite phase at a constant stress, thus producing a stress martenzita, nastalega iz avstenita [9]. Avstenit je stabilna faza v razmerah, ko ni napetosti. Če pa pride do kritičnih napetosti, avstenit popusti in se začne pri konstantni napetosti pretvarjati v martenzitno fazo tako, da nastane napetostna ploščad. Martenzit postane nestabilen, ko popustijo napetosti, zato se pri razbremenitvi martenzit ponovno pretvarja v avstenitno fazo in ko ni več nobene obremenitve, bi moral material imeti v celoti avstenitno zgradbo, tedaj vzorec dobi prvotno, nedeformirano obliko. Superelastičnost se pojavlja v temperaturnem območju $A_f < T < M_d$ (slika 4).

plateau. The martensite becomes unstable upon the removal of the stress; hence, during unloading, the martensite phase reverse transforms to the austenite phase and, at zero loads, the material should be entirely austenitic with the specimen recovering its original undeformed shape. Superelasticity typically occurs in a temperature range between Af < T < Md (Figure 4).



Slika 6. Shematični prikaz pojava superelastičnosti (krivulja napetost – deformacija za zlitino z oblikovnim spominom pri enoosni obremenitvi)

Figure 6. Schematic representation of superelastic effect (stress-strain curve for SMA under uniaxial loading).



Slika 7. Makroskopska in mikroskopska predstavitev superelastičnosti v zlitinah z oblikovnim spominom

Figure 7. Macroscopic and microscopic presentation of superelasticity in shape memory alloy

4 Eksperimentalne meritve

4.1 Meritve temperature A_f

V razmerah brez napetosti se temperature fazne premene navadno merijo z diferenčno vrstično kalorimetrijo (DSC). V naši raziskavi smo hoteli s to metodo ugotoviti temperaturo, pri kateri ima material (zlitina NiTi z oblikovnim spominom) popolnoma avstenitno mikrostrukturo in kaže superelastično obnašanje.

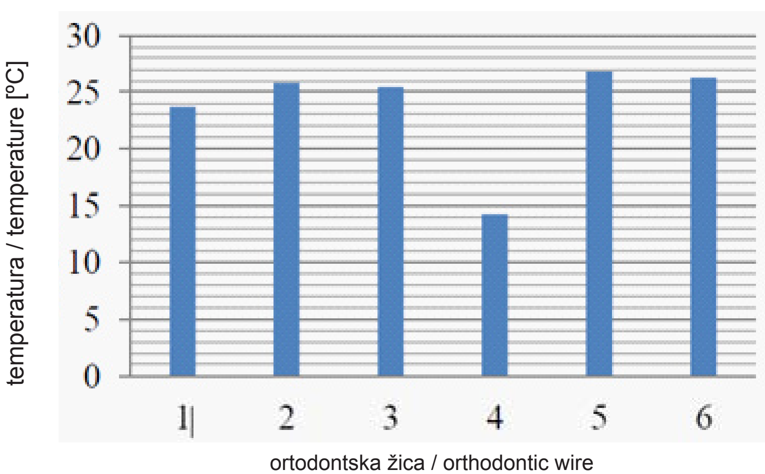
Pokazano je bilo, da imajo vse ortodontske žice temperaturo A_f pod telesno temperaturo. Temperature žic so

4 Experimental measurements

4.1 Measurements of A_f temperature

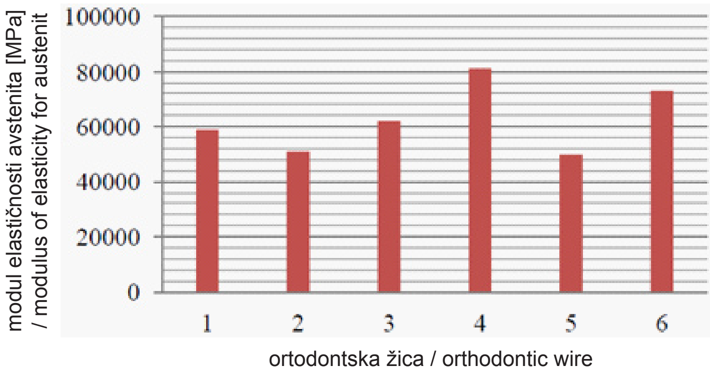
Under stress free conditions the phase transition temperatures are measured commonly by DSC (Differential Scanning Calorimetry). In our research we wanted with this method to find a temperature at which the material (SMA NiTi) has fully austenitic microstructure and shows superelastic behaviour.

It has been shown that all the orthodontic wires have the temperature A_f below body temperature. Wire temperatures were



Slika 8. Temperatura A_f za različne ortodontske žice

Figure 8. Temperature A_f for different orthodontic wires



Slika 9. Elastični modul avstenita za različne ortodontske žice.

Figure 9. Elastic modulus of austenite for different orthodontic wires

bile ugotovljene med 14 °C in 26 °C in to je prikazano na sliki 8. Deformacijska ploščad je močno odvisna od temperature A_f. Zato je temperatura A_f zlitine NiTi z oblikovnim spominom zelo pomembna pri vseh medicinskih uporabah.

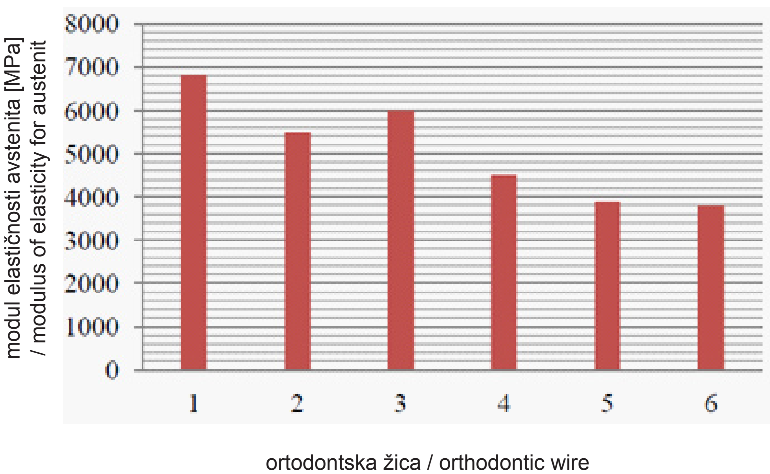
4.2 Meritve mehanskih lastnosti

Mehanske lastnosti zlitin z oblikovnim spominom so močno odvisne od temperature A_f. Naslednja razpredelnica kaže mehanske lastnosti ortodontskih žic iz zlitine NiTi z oblikovnim spominom, ugotovljenih z enoosnim nateznim preskusom. Za natezne preskuse smo uporabili stroj Zwick/Roell

analysed from 14 °C to 26 °C and are shown in Figure 8. The transformation plateau is highly dependent on the A^f temperature. Therefore, the A_f temperature of the SMA NiTi is very important in all medical applications.

4.2 Measurement of mechanical properties

The mechanical properties of SMA are largely dependent on the temperature Af. The following Table shows the mechanical properties of SMA NiTi orthodontic wires obtained from the uniaxial tensile test. The tensile tests were performed using a Zwick/



Slika 10. Elastični moduli različnih ortodontskih žic na premembni ploščadi

Figure 10. Elastic modulus of transformation plateau for different orthodontic wires

ZO 10. Krivulja napetost – deformacija pri statičnem nateznem preskusu je odvisna od mikrostrukture (avstenit, premena ali martenzit). Slika 9 kaže naklon začetnega dela obremenitvene krivulje (elastični modul avstenita). Moduli elastičnosti za avstenit so v območju 50 000 – 81 000 MPa.

Na sliki 10 je prikazan naklon premembne faze ali območja E_2 (prehod avstenita v martenzit) za različne ortodontske žice. Moduli elastičnosti za območje premene so med 4500 MPa in 6800 MPa.

Na sliki 11 je prikazan naklon zadnjega dela krivulje E_3 (elastični moduli martenzita) za različne ortodontske žice. Moduli elastičnosti martenzita so v območju 32 500 – 50 000 MPa.

Na sliki 12 so prikazani raztezki različnih ortodontskih žic na premembni ploščadi, l_t. Raztezki so v območju 5 – 7 %.

Na sliki 13 so prikazani raztezki l_s superelastičnosti za različne ortodontske žice. Ti raztezki so v območju 6 – 8 %. Ta podatek je zelo pomemben za ortodontsko žico, da deformacija pri ortodontski obdelavi ne preseže tega območja.

Na sliki 14 so prikazani raztezki povratne elastičnosti lsb za različne ortodontske žice. To pomeni stopnjo, do katere se aktivirana žica loka vrne v prvotno stanje po dezaktiviranju [10]. Ti so v območju 10 –

Roell ZO 10. The stress-strain curve from a static tensile test depends on microstructure (austenite, transformation or martensite).

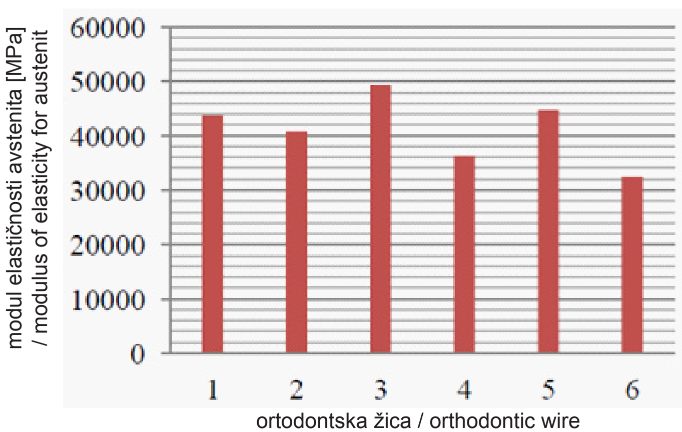
In Figure 9 are presented the slope of the initial part of the loading curve (elastic modulus of austenite). Modules of elasticity for austenite are in the range from 50000 to 81000 MPa.

Figure 10 presents the slope of the transformation phase or region E_2 (austenite to martensite) for different orthodontic wires. Modules of elasticity for the transformation region are in range from 4500 to 6800 MPa.

Figure 11 presents the slope of the final part of the curve E_3 (elastic modules of martensite) for different orthodontic wires. Modules of elasticity for martensite are in the range from 32500 to 50000 MPa.

Figure 12 presents the elongations of transformation plateau lt for different orthodontic wires. These elongations are in the range from 5 to 7 %.

Figure 13 presents ls the elongation of superelasticity for different orthodontic wires. These elongations are in the range from 6 to 8 %. This information is very important in orthodontic wire, because deformation in orthodontic treatment does not exceed this range.

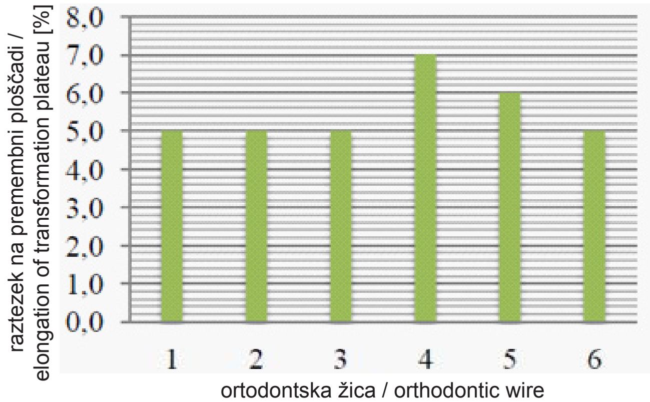


Slika 11. Elastični modul martenzita za različne ortodontske žice

Figure 11. Elastic modulus of martensite for different orthodontic wires

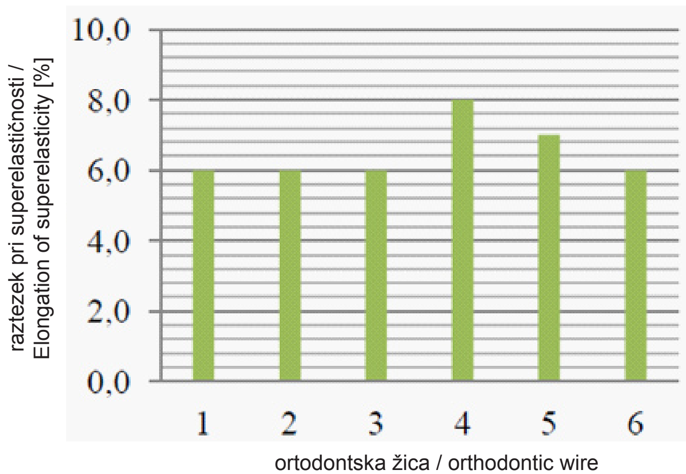
Slika 12. Raztezki različnih ortodontskih žic na premembni ploščadi

Figure 12. Elongation of transformation plateau for different orthodontic wires



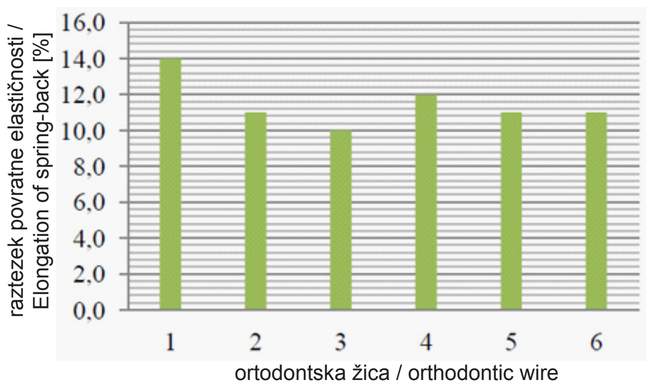
Slika 13. Raztezek pri superelastičnosti za različne ortodontske žice

Figure 13. Elongation of superelasticity for different orthodontic wires



Slika 14. Raztezek povratne elastičnosti za različne ortodontske žice

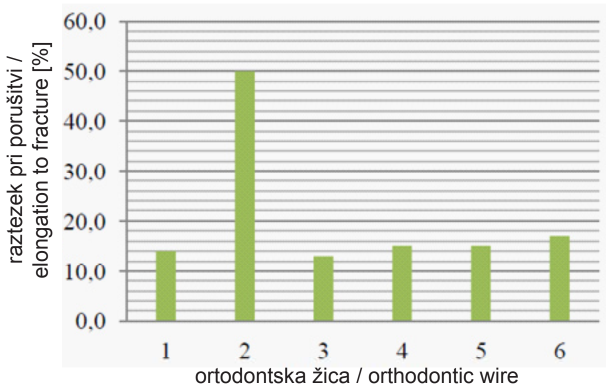
Figure 14. Elongation of spring-back for different orthodontic wires



14 %. Ta podatek je pomemben, da se zna načrtovati sestavine za različne uporabe.

Slika 15 prikazuje raztezke pri porušitvi, l_f, ortodontskih žic. Ti raztezki so v območju

Figure 14 presents the elongations of spring back l_{sb} for different orthodontic wires. This means the extent to which the range recovers upon deactivation of an activated



Slika 15. Raztezki pri porušitvi za različne ortodontske žice

Figure 15. Elongation to fracture for different orthodontic wires

13 – 50 %. Ortodontska žica 2 je imela za razliko od ostalih 50 %-ni raztezek.

5 Biokompatibilnost

Splošno privzeta definicija biokompatibilnosti je zelo zapletena. Williams (1988) je biokompatibilnost opisal kot »sposobnost materiala, da deluje s primernim odzivom gostitelja pri določeni uporabi«. Ta definicija pokrije dejstvo, da je možnih veliko interakcij med biomaterialom in gostiteljem ter da se ne osredotoča samo na učinke zastrupitve ali poškodbe gostiteljevega sistema. Bolj konkretno definicijo je leta 1998 predlagal Wintermantel, ki je biokompatibilnost definiral kot strukturno in površinsko kompatibilnost med tehničnim in biološkim sistemom, kjer strukturni del določajo mehanske lastnosti, površinsko kompatibilnost pa določajo površinske fizikalne, kemične, biološke in morfološke lastnosti biomateriala. Danes je potrebno biokompatibilnost vrednotiti z ustreznimi standardi [11].

5.1 Vpliv kemičnih elementov niklja in titana

Titan se smatra kot zelo biokompatibilen. Na drugi strani je nikelj biokompatibilen in strupen. Nikelj je zelo močan alergen

arch-wire [10]. This size is in the range from 10 to 14 %. This information is important so as to know the design components for a variety of applications.

Figure 15 presents the elongations to fracture l_f of orthodontic wires. These elongations are in the range from 13 to 50 %. Orthodontic wire 2 has, unlike the others, 50% of elongation to fracture.

5 Biocompatibility

The generally accepted definition of biocompatibility is very complicated. Williams (1988) described biocompatibility as "the ability of a material to perform with appropriate host response in a specific application". This definition covers the fact that there are a variety of interactions possible between biomaterial and the host, and it does not focus solely on toxicity or injurious effects to the host system. A more concrete definition was proposed in 1998 by Wintermantel, who defined biocompatibility as the structural and surface compatibility between a technical and a biological system, where the structural part is governed by the mechanical properties, and the surface compatibility by the biomaterial's superficial physical, chemical, biological and morphological properties. Today, it is in povzroča zelo občutljivo reakcijo v večji meri kot katerakoli kovina ali zlitina. Kovinski nikelj, nikljev sulfid, nikljev oksid in nikljev karbonat so za človeka zanesljivo karcerogeni [12].

5.2 Zaščitna plast TiO_2

Titan in njegove zlitine lahko nastanejo na zunanji površini filma titanovega oksida (TiO_2) z majhnim deležem NiO. Zaradi sposobnosti tvorbe plasti stabilnega titanovega oksida je titan eden od najbolj biokompatibilnih materialov. V najugodnejših razmerah, ki predstavljajo izvrstno osteo integracijo s kostjo, je tudi sposobnost tvorbe kalcijevega fosfata na površini, ki tudi preprečuje korozijo. Druga koristna lastnost je, da se v primeru poškodbe zaščitne plasti, predvsem titanovega oksida in kalcijevega fosfata, ta plast regenerira [13].

Cilj prve faze ocene biokompatibilnosti žice iz Ni-Ti je preiskava možne citotoksičnosti ortodontskih žic iz Ni-Ti na modelu podganjih timocitov, kjer se ustvari neposreden stik med materialom in celicami.

Ta preskus je bil izbran zato, ker je velika občutljivost timocitov na pro-apoptotične dražljaje in preskus vključuje kulture celic, ki se ne zraščajo in ne razmnožujejo, kot so timociti, in je mnogo primernejši za oceno citotoksičnosti materiala v neposrednem stiku v primerjavi z zraščenimi celicami, kot so fibroblasti.

Poskus je bil pripravljen tako, da se je pripravilo gojišče podganjih timocitov z vloženimi vzorci Ni-Ti-žice pri različnih razmerjih površina-prostornina žic v celotnem mediju. Citotoksični učinek se je ocenjeval z meritvami apoptoze.

Timociti so bili izolirani iz timusov (priželjcev) moških podgan albino oxford (AO), starih 2-2 in pol mesecev. Vse študije na živalih je odobril odbor za etiko inštituta

necessary to evaluate the biocompatibility in accordance with the relevant Standard [11].

5.1 The influence of the chemical elements nickel and titanium

Titan is classified as highly biocompatible. On the contrary, the nickel is biocompatible and toxic. Nickel is a very strong allergen and causes a very sensitive reaction to a greater extent than any metal or alloy. Nickel metal, nickel sulphide, nickel oxide and nickel carbonate are definitely human carcinogens [12].

5.2 Protective layer of TiO_2

The titanium and its alloys can be formed on the outer surface of the film of titanium oxide (TiO_2) with a small amount of NiO. Due to the ability to form a stable titanium oxide layer on the surface, this is one of the most biocompatible materials. In the optimum condition, which can be an excellent osteo-integration with the bone, there is also the ability to form calcium phosphate on the surface, which also prevents corrosion. Another useful property is that, in the event of damage to the protective layer, in particular titanium oxide and calcium phosphate, the layer can be regenerated [13].

The aim of the first phase of biocompatibility assessment of Ni-Ti wire was to investigate the potential cytotoxicity of Ni-Ti orthodontic wires on a model of rat thymocytes, where a direct contact exists between the material and the cells.

The test was chosen because of the high sensitivity of thymocytes to pro-apoptotic stimuli and because the test includes the culture of non-adherent, non-proliferating cells, such as thymocytes, which is much more convenient for the evaluation of a material's cytotoxicity in direct contact, za medicinske raziskave v skladu z navodili in priporočili EU za varovanje laboratorijskih živali. Po evtanaziji živali v etru se je kirurško s skalpelom odstranil timus in sosednje tkivo okoli timusa. Timus se je potem homogeniziral v najlonski mrežici s sponko v obliki brizge in suspenzija celic se je pripravila v RPMI-mediju. Sledilo je filtriranje in izpiranje suspenzije celic s centrifugo z 1400 obr./min 8 minut. Kepica celic se je ponovno suspendirala v popolnem RPMI-mediju, ki je bil sestavljen iz 10 % seruma iz plodu telička (FCS), 2-merkaptoetanola (200 μM) in antibiotikov (gentamicin, penicilin in streptomicin, 1 % raztopina). Preživetje celic po taki izolaciji je bila vedno večje od 95 %, kot ugotovljeno z obarvanjem celic z raztopino Trypan modro.

5.3 Priprava Ni-Ti-žic za biokompatibilnostne preskuse

Ni-Ti-žice (0,46 mm x 0,76 mmx 1000 mm), s površino 0,25 cm^2 za posamezno žico, so bile oprane v sterilni vodi in obdelane z ultrazvokom 10 minut pri sobni temperaturi. Vodna raztopina je bila potem zamenjana z 50 %-nim etilnim alkoholom in sledila je nadaljnja 15-minutna obdelava z ultrazvokom. Končno smo to raztopino zamenjali z 96 %-nim alkoholom in vzorce znova obdelovali 15 minut z ultrazvokom. Nato smo vzorce dali z diamantno pinceto v petrijevke, izpostavili laminarnemu toku zraka in za 10 ur UV-svetlobi. Tako sterilizirane vzorce ortodontnih žic iz Ni-Ti smo uporabili za preskuse biokompatibilnosti in kondicioniranja.

5.4 Gojenje timocitov in razmere za kondicioniranje

Timocite (4 x 10^6 celic na petrijevko) smo gojili v 24 petrijevkah v popolnem RPMI-mediju (500 μl na petrijevko) ali same ali s

compared to the adherent cells such as fibroblasts.

The experiment was designed to cultivate the Ni-Ti wire samples with rat thymocytes, using different surface-over-volume ratios of the wires in complete medium. The cytotoxic effect was evaluated by measuring the apoptosis.

Thymocytes were isolated from the thymuses of Albino oxford (AO) male rats, 2-2.5 months old. All studies on animals were approved by the Ethics Committee of the Institute for Medical Research, which subjugates the EU Guidelines and Recommendations on Laboratory Animal Welfare. Following the euthanasia of animals in ether and the surgical extraction of the thymus, the connective tissue around the thymus was removed using a scalpel. The thymus was then homogenized over a nylon mesh with a syringe clip and the cell suspension was made in basic RPMI medium. This was followed by filtration and washing of the cell suspension by centrifugation at 1400 rpm for 8 minutes. The cell pellet was re-suspended in complete RPMI medium, which consisted of 10% foetal calf serum (FCS), 2-mercaptoethanol (200 uM) and antibiotics (gentamicin, penicillin and streptomycin, 1% solution). The cells' viability after such isolation was always higher than 95%, as determined by staining the cells with Trypan blue solution.

5.3 Preparation of Ni-Ti wires for biocompatibility tests

Ni-Ti wires (0.46 x 0.76 x 1000 mm) with the surface of 0.25 cm^2 per wire were washed in sterile water and sonified at room temperature for 10 minutes. The aqueous solution was then replaced with 50% ethyl alcohol and the sonification continued for another 15 minutes. Finally, the solution was prisotnimi ortodontskimi žicami. Ali pa smo celice gojili skupaj s steklenimi paličicami, ki niso bile citotoksične, kot smo preverili predhodno. Površina vzorcev orto Ni-Ti v celotni prostornini gojitvenega medija (razmerje površina-prostornina) se je gibala od 0,5 cm^2/ml do 12,0 cm^2/ml. Celice so se gojile pri 37 °C in 5 % CO_2 24 ur, nato smo napravili citotoksične preskuse.

Ortodontske žice iz Ni-Ti smo kondicionirali v 3 ml popolnega RPMI-medija v 6 petrijevkah. Površina žice glede na prostornino medija je bila 3 cm^2/ml. Po 7 dneh kondicioniranja smo medije zbrali in jih med prenosom vzorcev na analizo zamrznili pri -20 °C. Vzorce Ni-Ti smo prekrili z enako prostornino svežega medija. Da bi ugotovili učinke kondicioniranega medija na površinsko mikrostrukturo Ni-Ti-vzorcev smo žice zbrali po 7 dneh kondicioniranja in jih 10 minut prali z ultrazvokom v sterilni vodi in nato sušili v laminarnem toku ter jih shranili v sterilnih Eppendorfovih epruvetah. Drugi del vzorcev je bil shranjen v Eppendorfovih epruvetah brez predhodnega splakovanja.

5.5 Ocene citotoksičnosti

Po 24 h smo z morfološko metodo ugotavljali apoptozo timocitov, ki so bili gojeni v prisotnosti ortodontskih žic iz Ni-Ti, s tem, da smo celice obarvali s Turkovo raztopino. Apoptotične celice smo ugotovili na osnovi njihovega homogeno obarvanega heterokromatina in delež apoptotičnih celic smo izračunali na osnovi vsaj 500 celic.

Dodatno smo ugotavljali apoptozo timocitov, gojenih v prisotnosti Ni-Ti-žic, s pretočno citometrijo, ko smo celice označili s propidijevim jodidom (PI), raztopljenim v hipotonični raztopini. Analizo smo naredili s pretočnim citometrom EPICS XL-MCL (Coulter, Krefeld, Nemčija) z 488 nm vzbujalnim laserjem. Med analizo je bil prehod timocitov krmiljen glede na njihovo

replaced with 96% alcohol, and the samples were sonified for another 15 minutes. The samples were then placed on Petri dishes using diamond tweezers, placed under a laminar air flow and exposed to UV light for 2-10 hours. The sterilized samples of Ni-Ti Orthodontic wires were then used in the tests of biocompatibility and conditioning.

5.4 Cultivation of thymocytes and terms of conditioning

Thymocytes (4 x 10^6 cells/well) were cultured in a 24-well plate in complete RPMI medium (500 ul/well), either alone, or in the presence of orthodontic Ni-Ti samples. Alternatively, the cells were cultivated with control glass rods, which are not cytotoxic, as we found previously. The area of Ortho Ni-Ti samples in the total volume of the culture medium (surface-to-volume ratio) ranged from 0.5 cm2/ml to 12.0 cm^2/ml. The cells were cultured at 37 °C and 5% CO2 for 24 hours, after which cytotoxicity tests were carried out.

Ni-Ti orthodontic wires were conditioned in 3 ml of complete RPMI medium in 6-wells plates. The surface of alloy over the volume of medium was 3cm^2/ml. After 7 days of conditioning the media were collected and frozen at -20 °C, until the transportation of samples for the analysis. The other Ni-Ti samples were covered with the same volume of fresh medium. In order to determine the effects of conditioned medium on the surface microstructure of Ni-Ti samples, the wires were collected after 7 days of conditioning and washed by sonification in sterile water for 10 minutes, followed by drying in a laminar flow and preservation in sterile Eppendorf tubes. The second part of the samples was preserved in Eppendorf tubes without prior rinsing.

velikost in zrnavost z računalniškim programom na osnovi diagrama FS x SS, narejenega v SISTEMU II™ . Dodatno analizo smo napravili s programom FlowJo. Rezultati iz pretočnega citometra so predstavljeni grafično, prikazujejo apoptotične celice, ki so bile označene kot celice z manjšo fluorescenco in primerjane z živimi celicami, glede na zavrženi genetski material. Raven nespecifične fluorescence je bila ugotovljena z vzorci, ki niso bili obarvani z PI.

5.6 Učinek Ni-Ti žic na citotoksičnost in vitro

Te študije smo izbrali, ker so timociti (nedozoreli timični T-limfociti) občutljivi za apoptotične signale in vitro in glede na literaturne podatke o pro-apoptotičnem učinku Ni-ionov. Uporabili smo najmanjše (0,5 cm^2/ml) in največje (6,0 cm^2/ml) razmerje med površino zlitine in prostornino medija s celično kulturo pri analizi z neposrednim stikom med celicami in materialom. Kot negativne primerjalne vzorce smo uporabili vzorce laboratorijskih steklenih paličic,

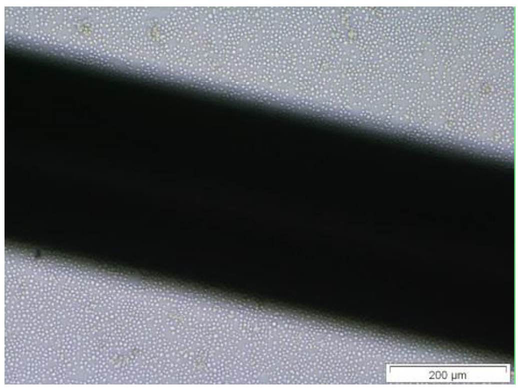
Slika 16 Prikaz timocitov, ki so bili gojeni 24 h v popolnem gojitvenem mediju

Figure 16. The appearance of control thymocytes cultivated for 24 h in complete culture medium.

5.5 Cytotoxicity assays

After 24 h cultivation, the apoptosis of thymocytes cultivated in the presence of Ni-Ti orthodontic wires was determined using the morphological method, by staining the cells with Turk solution. Apoptotic cells were detected on the basis of their homogeneously stained heterochromatin, and the percentage of apoptotic cells was calculated on the basis of at least 500 cells.

Additionally, the apoptosis of thymocytes cultivated in the presence of Ni-Ti wires was determined using flow cytometry, upon labelling the cells with propidium iodide (PI) dissolved in hypotonic solution. The analysis was performed on the flow cytometer EPICS XL-MCL (Coulter, Krefeld, Germany) using a 488 nm excitation laser. During the analysis, thymocytes were gated based on their size and granularity, using the FS x SS diagram created in the SYSTEM II ™ software programme. Subsequent analysis was performed using FlowJo software. The



Slika 17. Prikaz timocitov, ki so bili gojeni 24 h v popolnem gojitvenem mediju ob prisotnosti Ni-Ti-vzorcev

Figure 17. The appearance of thymocytes cultivated for 24 h in complete culture medium in the presence of Ni-Ti samples

obdelanih z raztopino deksametazona, ki je poznan po svojem farmakološkem uravnavanju apoptoze. Pojav kultur timocitov v prisotnosti ortodontskih žic iz Ni-Ti prikazujeta sliki 16 in 17.

Rezultati, ki se nanašajo na morfološko analizo, so prikazani na sliki 18.

Po 24 h je bilo gojišče z Ni-Ti-žicami ali paličicami za primerjanje in timociti obarvano s Turkovo raztopino in preiskano s svetlobno mikroskopijo. Apoptotične celice se je ugotovilo s homogeno obarvanim heterokromatinom, medtem ko je bil delež apoptotičnih celic izračunan na osnovi analize vsaj 500 celic. Rezultati so pokazali, da je bila srednja vrednost ±SD (n = 5) pri enem od reprezentativnih poskusov *p<0,05, ***p<0,05 v primerjavi z ustreznimi primerjalnimi vzorci (primerjalne steklene paličice).

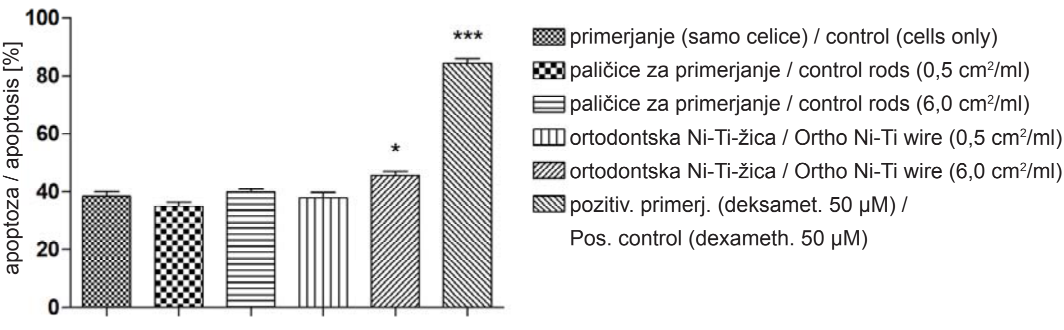
Sorazmerno velik delež apostoze je bil ugotovljen v gojišču, ki ni bilo občutno spremenjeno ali z negativnimi primerjalnimi vzorci ali zaradi vpliva vzorcev Ni-Ti ortodontskih žic z manjšo površino (0,5 cm²/ml). A Ni-Ti žice z večjo površino so v enaki prostornini medija (6,0 cm²/ml) občutno povečale apoptozo. Odstotek apoptotičnih timocitov je bil občutno manjši v primerjavi s pozitivnimi primerjalnimi vzorci in ta učinek

results from the flow cytometry are presented graphically, showing the apoptotic cells that were marked as the cells with a lower fluorescence compared to the live cells, due to their discarded genetic material. The level of nonspecific fluorescence was determined based on the samples that were not stained with PI.

5.6 The effect of Ni-Ti wires on cytotoxicity in vitro

These studies were chosen because of the sensitivity of thymocytes (immature thymic T lymphocytes) to apoptotic signals in vitro and literature data on the pro-apoptotic effect of Ni ions. We used the lowest (0.5 cm²/ml) and the highest (6.0 cm²/ml) alloys' surface-to-volume ratio in cell culture medium, in an assay of direct contact between the cells and the material. As a negative control we used the samples of laboratory glass rods with the same surface-to-volume ratio, whereas positive control samples were treated with a dexamethasone solution, which is known for its pharmacological modulation of apoptosis. The appearance of the thymocytes' cultures with Ni-Ti orthodontic wires, or control cultures is shown in Figures 16 and 17.

The results considering morphological analysis are presented in Figure 18.



Slika 18 Morfološka analiza apoptoze timocitov v gojišču skupaj z Ni-Ti-zlitinami

Figure 18. Morphological analysis of thymocytes' apoptosis in culture with Ni-Ti alloys

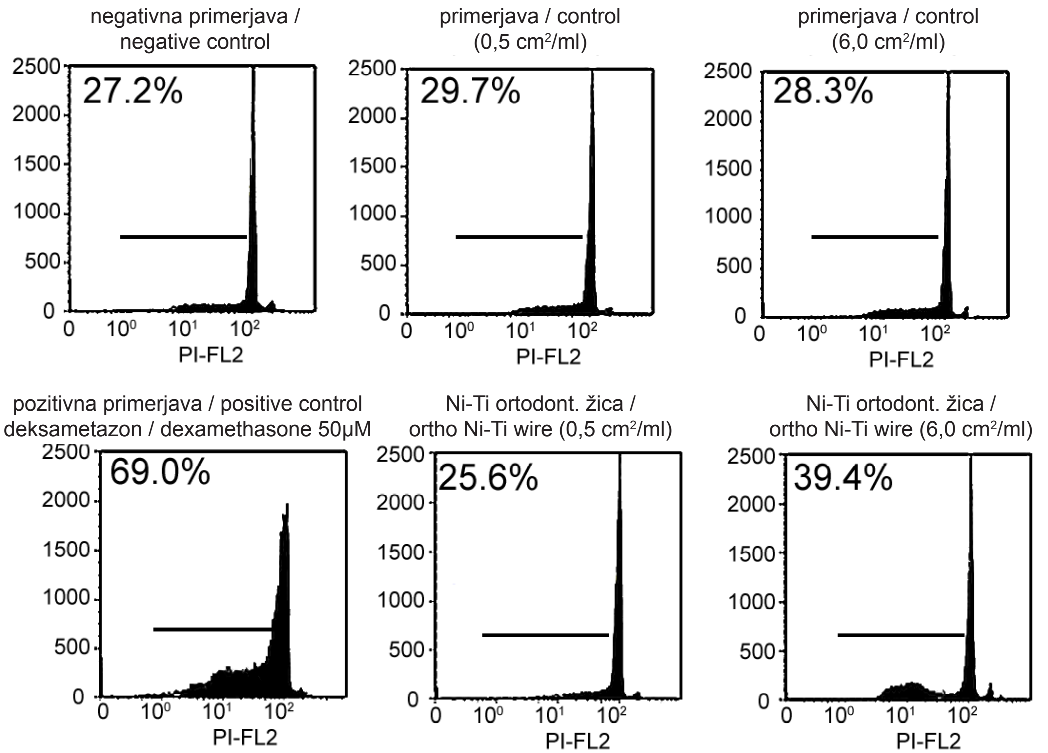
se lahko opiše kot šibek pri ocenjevanju po lestvici šibko – zmerno – močno.

Podoben pro-apoptotični učinek Ni-Ti-žic je bil potrjen pri drugi analizi apoptoze timocitov z obarvanjem jeder s propidijevim jodidom. Slika 19 prikazuje reprezentativni preskus, medtem ko slika 20 kaže srednjo vrednost treh poskusov. Rahlo manjši odstotek celic s hipodiploidnimi jedri je v soglasju z že opisanim mehanizmom dinamičnega razvoja apoptoze v gojišču celic.

Po 24 h (Fig 19) je bilo gojišče timocitov skupaj z Ni-Ti-žicami ali primerjalnimi paličicami obarvano s propidijevim jodidom in analizirano s pretočnim citometrom. Apoptotične celice se je ugotavljalo na osnovi manjše količine genetskega materiala in s tem manjše intenzivnosti fluorescence

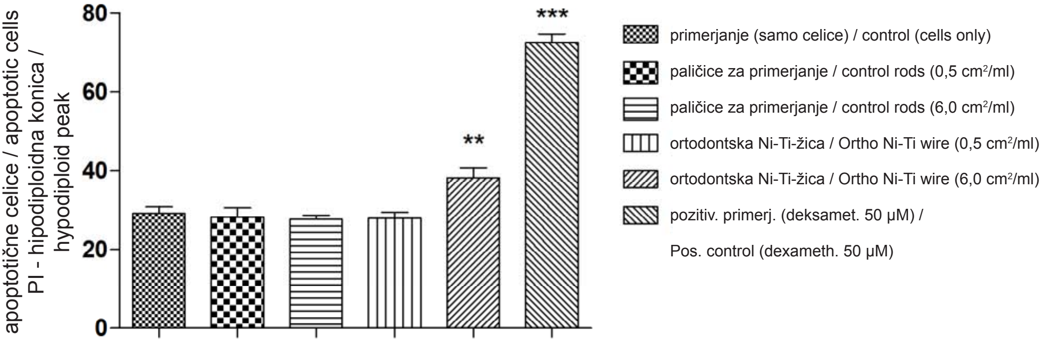
After 24 h – culture with Ni-Ti wires or control rods, thymocytes were stained using Turk solution and analysed by light microscopy. Apoptotic cells were detected on the basis of homogenously stained heterochromatin, whereas the percentage of apoptotic cells was calculated by analysing at least 500 cells. The results are shown as mean ± SD (n = 5) from one representative experiment. *p<0.05, ***p<0.005, compared to corresponding control samples (control glass rods).

There was a relatively high rate of apoptosis in the culture, which was not modified significantly either by the negative control samples nor by the influence of orthodontic Ni-Ti samples of smaller surface area (0.5 cm^2 / ml). However, the larger surface of Ni-Ti wires that was placed



Slika 19. Analiza apoptoze timocitov v gojišču skupaj z Ni-Ti žicami s pretočnim citometrom.

Figure 19. Analysis of thymocytes' apoptosis in culture with Ni-Ti alloys by flow cytometry.



Slika 20. Analiza apoptoze timocitov v gojišču skupaj z Ni-Ti žicami s pretočnim citometrom

Figure 20. Analysis of thymocytes' apoptosis in culture with Ni-Ti alloys by flow cytometry

kot hipodiploidne celice. Reprezentativni histogram predstavlja en reprezentativni poskus.

Po 24 h je bilo gojišče timocitov skupaj z Ni-Ti-žicami ali primerjalnimi paličicami obarvano s propidijevim jodidom in analizirano s pretočnim citometrom. Apoptotične celice se je ugotavljalo na osnovi manjše količine genetskega materiala in s tem manjše intenzivnosti fluorescence kot hipodiploidne celice. Rezultati so prikazani kot srednja vrednost ± SD treh poskusov **p<0,01, ***p<0,05, v primerjavi z ustreznimi primerjalnimi vzorci (primerjalne steklene paličice).

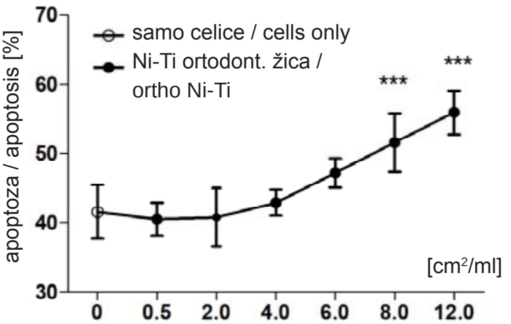
V drugem poskusu smo preiskovali v odvisnosti od doziranja učinek Ni-Ti ortodontskih žic na apoptozo podganjih timocitov v gojišču. Rezultate prikazuje slika 21. Vidi se, da je v okviru razmerja površina – prostornina biomateriala v mediju, ki ga priporočajo standardi ISO (0,5 – 6 cm^2/ml) samo večja količina Ni-Ti-žic povečala apoptozo timocitov, kar je občutno več kot pri spontani hipoptozi v gojišču. Količina Ni-Ti-žic, ki je bila večja od priporočene (8,0 cm^2/ml in 12,0 cm^2/ml) je še dodatno povečala apoptozo vendar brez večje odvisnosti od doziranja.

in the same volume of medium (6.0 cm^2/ml) increased apoptosis significantly. The percentage of apoptotic thymocytes was lower significantly compared to the positive control, and this effect can be assessed based on the weak-moderate-strong scale, as a weak one.

A similar pro-apoptotic effect of Ni-Ti wires was confirmed in another assay of thymocytes' apoptosis, by staining the nuclei with propidium iodide. Figure 19 shows a representative experiment, whereas Figure 20 shows the mean of three experiments. The slightly lower percentage of cells with hypodiploid nuclei is in line with the already described˝mechanism of dynamic development of apoptosis in cell culture.

After 24 h (Fig 19) culture with Ni-Ti wires or control rods thymocytes were stained with propidium iodide and analysed by flow cytometry. Apoptotic cells were detected based on the smaller amount of genetic material, and thus the lower fluorescence intensity, as hypodiploid cells. Representative histograms are shown from one representative experiment.

After 24 h (Fig. 20) culture with Ni-Ti wires or control rods, thymocytes were stained with propidium iodide and



Slika 21. Učinek razmerja površina Ni-Ti-žic in prostornina medija, v odvisnosti od doziranja, na apoptozo timocitov v gojišču

Figure 21. Dose-dependent effect of Ni-Ti alloy's surface-to-volume of medium ratio on the apoptosis of thymocytes in culture

Po 24 h gojenja skupaj z Ni-Ti-žicami ali primerjalnimi paličicami so bili timociti obarvani s Turkovo raztopino in pregledani s svetlobno mikroskopijo. Apoptotične celice so bile ugotavljane s homogeno obarvanim heterokromatinom, medtem ko je bil delež apoptotičnih celic izračunan na osnovi analize vsaj 500 celic. Rezultati so prikazani kot srednja vrednost ± SD (n = 5) enega od reprezentativnih poskusov *p<0,05, ***p<0,005, v primerjavi z ustreznimi primerjalnimi vzorci (primerjalne steklene paličice).

6 Sklepi

Prispevek predstavlja funkcionalne lastnosti zlitin NiTi, zaradi katerih se uporabljajo v ortodontski praksi. Iz poznavanja mehanskih lastnosti ortodontskih žic lahko predvidimo, katera vrsta žic bo povzročala večje oziroma manjše sile na zobeh med ortodontskim zdravljenjem. Mehanske lastnosti žic so v veliki meri odvisne od procesa izdelave, ki je zapleten in sestavljen iz velikega

analysed by flow cytometry. Apoptotic cells were detected based on the smaller amount of genetic material, and thus lower fluorescence intensity, as hypodiploid cells. Result are presented as mean ± SD of three experiments **p < 0.01, ***p < 0.005, compared to corresponding control samples (control glass rods).

In another experiment we investigated a dose-dependent effect of Ni-Ti orthodontic wires on the apoptosis of rat thymocytes in culture. The results are shown in Figure 21. It was shown that, within the surface-to-volume ratio of biomaterial in a medium that is recommended by ISO Standards (0.5 - 6 cm2/ml), only the highest amount of Ni-Ti wires increased apoptosis of thymocytes, which was higher significantly compared to spontaneous apoptosis in culture. The amount of Ni-Ti wires that was higher than the recommended one (8.0 and 12.0c m2/ml), increased apoptosis additionally, but without significant dose-dependent effect.

After 24h-culture with Ni-Ti wires or control rods, thymocytes were stained using Turk solution and analysed by light microscopy. Apoptotic cells were detected on the basis of homogenously stained heterochromatin, whereas the percentage of apoptotic cells was calculated by analysing at least 500 cells. The results are shown as mean ± SD (n = 5) from one representative experiment. *p < 0.05, ***p < 0.005, compared to corresponding control samples (control glass rods).

6. Conclusions

The paper presents the essential features which make NiTi alloy used in orthodontic practice. By mechanical tests on orthodontic wires it is possible to predict which wire will cause a greater or lesser force on the tooth during orthodontic treatment.

števila različnih operacij. Pri tem obstajajo omejitve, ki so vezane na fazo hladnega preoblikovanja. Seveda pa je pri celovitem obravnavanju ortodontskih žic potrebno poznati tudi biološke vplive žic na človeško telo, saj so ti lahko škodljivi. Pri tem si lahko pomagamo s preskusi biokompatibilnosti. Zaradi posebnih lastnosti NiTi zlitine se te uporabljajo na različnih področjih (medicina, letalstvo, vesoljska tehnologija, rekreacija). V prihodnosti pričakujemo uporabo teh zlitin na novih področjih, saj kot je predstavljeno v članku, lahko dosežemo različne mehanske lastnosti kljub omejitvam pri izdelavi (hladno oblikovanje).

Po drugi strani so preskusi biokompatibilnosti pokazali, da imajo vzorci Ni-Ti ortodontskih žic šibek citotoksičen učinek (indukcija apoptoze) na podganje timocite v gojišču, in to le za primer, ko je bilo uporabljeno največje razmerje površine zlitine in prostornine medija (6 cm^2/ml), ki je priporočeno z ISO standardom za preskušanje biokompatibilnosti.

However, it is important to know which wire has adverse effects on the human body, so it is necessary to perform a biocompatibility test. The mechanical properties of single wires have a significant impact on the production process of wires. It is a very complex process that requires a lot of different operations. Due to their specific characteristics SMA NiTi are already used in many different applications (medicine, aviation, space technology, recreation). However, it can be expected in the future in many new applications of these alloys because, as we present, the article may achieve different mechanical properties, although there are limits to the actual manufacture of these alloys (cold forming).

On the other hand, testing of biocompatibility presents that samples of Ni-Ti wires showed mild cytotoxic effect (induction of apoptosis) on rat thymocytes in culture, only when applied with the highest surface-to-volume ratio of alloy in medium (6 cm^2/ml) that is recommended by ISO Standards for biocompatibility testing.

7 Viri / References

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